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MEDICAL RESEARCH COUNCIL

REPORTS ON BIOLOGICAL STANDARDS

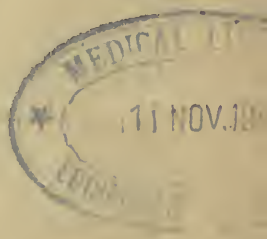
I. On the Physiological Standardization of Extracts of the Posterior Lobe of the Pituitary Body

BY

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AND

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INTRODUCTION

It has been part of the consistent policy of the Council to promote researches which have as their object the attainment of well-based methods of estimating the quantitative and qualitative values of biological substances used either in medical research work itself or in the preventive and curative work of the medical profession. The investigations of this kind which they have supported from public funds are chiefly centred at their National Institute for Medical Research, at Hampstead, which in that respect is designed to fulfil in the biological sphere of medicine functions analogous to those of the National Physical Laboratory. So far as means and men are available, there are being actively prosecuted here systematic studies of biological methods of measurement and assay necessary for the determination of 'standards' of value, in the absence of which the scientific progress of medicine has been, and still is being, greatly hampered. It is the design of the Council to publish the results of work of this kind as they become available, and they hope that the Report now presented may be only the first of an extended series.

The importance to the public and to the medical profession of the subject treated in the present Report is very great, and no words need be added to enforce the grave observations made here by Dr. Dale as the outcome of his work with Dr. Burn. Extracts of the pituitary gland are used widely and increasingly in medical and surgical practice throughout the country. They provide, in particular, a remedy which, in the various difficulties or accidents of childbirth, is at once highly valuable and, like many other valuable remedies, often dangerous. Rightly used, it is a weapon in the hands of the doctor by which he may avoid difficulties or save life; wrongly or inaccurately used, the dangers it brings to mother and child may be extreme. Both the benefits and the dangers which this drug can bring must obviously depend chiefly upon the factor of dose, and the experiments described in this Report show that the commercial preparations used by medical practitioners, and naturally, or at least habitually, regarded by them as equivalent to one another, may actually so vary that the most potent may have 80 times the strength of the least potent. A doctor accustomed to one preparation may, when forced to use its apparent equivalent in an emergency, be under danger therefore of giving, 80 times the intended dose, and the most serious or even fatal consequences may follow.

It is clear that the manufacturers are by no means chiefly to blame for the position which is displayed so clearly here. It is pointed out that no official guidance has been given to them in this country as to the strength of extract most suitable for therapeutic use, and no standard of activity has been available for common reference, though all the extracts examined at the National Institute were described as being physiologically standardized. No dissent is possible from Dr. Dale's view that the present position is 'deplorable'.

It is a position, however, which fortunately is quite unnecessary and easily to be avoided. The United States Pharmacopoeia has officially laid down a theoretical strength for this extract. This Report recommends the adoption of the same theoretical strength for this country, and indicates a method by which a preparation of uniform activity can be made and used as a standard of reference. It may be noted that the British Pharmacopoeia has not yet recognized the existence of this potent and common remedy.

It is earnestly hoped that early action may be taken as the result of representations made in detail by the Medical Research Committee in 1916 to Mr. Asquith's Reconstruction Committee, and later in 1919 to the President of the Local Government Board (subsequently the Minister of Health), upon the urgent need for official standards of value and authenticity for drugs of this kind and for the numerous biological preparations, sera, and the like, used in medical practice. The Committee on those occasions expressed their conviction that the absence of the possibilities of control which would be given by the determination and imposition of standards was 'discreditable to our national position in the world of science and a source of grave danger to the community'. The Departmental Committee appointed to advise the Minister of Health upon this subject in April, 1920, has already reported strongly in favour of immediate measures, and has formulated a detailed scheme. (Command paper 1156, 1921, Stationery Office, price 2d.)

In the meantime, a Committee appointed jointly by the Obstetrical Section of the Royal Society of Medicine and the Medical Research Council is conducting a thorough investigation into the doses of pituitary extract appropriate for various medical and surgical uses, and more particularly for administration to women in labour. The Council agree with Dr. Dale that 'it will be truly lamentable if the results of this important inquiry, when in due course they are published, should be deprived of all accurate meaning by the failure of the manufacturers to bring all the makes of pituitary extract to such a uniform standard of activity as, without serious trouble, could be ensured'.

MEDICAL RESEARCH COUNCIL,
15 YORK BUILDINGS,
ADELPHI,
LONDON, W.C. 2.

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ON THE PHYSIOLOGICAL STANDARDIZATION OF EXTRACTS OF THE POSTERIOR LOBE OF THE PITUITARY BODY

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(From the National Institute for Medical Research, Hampstead)

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I. INTRODUCTION.

MUCH has been written in the past two decades concerning the necessity for employing a physiological method for measuring, and adjusting to a common standard, the activity of potent remedies, when the active principles are unknown, or of such a nature that they cannot be estimated by chemical methods. In no case is the necessity less open to discussion than in that of the now widely used watery extract of the posterior lobe of the pituitary body. The active principles are unknown and what little is known as to their nature indicates that they are very unstable, so that they are easily destroyed in the process of preparing the extract. The extract is essentially a remedy for emergencies. There are physicians who discount the value of physiological standards for the activity of digitalis, for example, on the ground that the real test is made in each case, by determining the reaction of the individual patient to the tincture which happens to be in use. The obstetrical physician faced with a post-partum haemorrhage, or the surgeon called to deal with a post-operative paralysis of the bowel, cannot take time to discover by experiment what dose, if any, of a particular sample of the pituitary extract will produce an effect sufficient to save the patient's life. Abundant proof has already been produced to show that the commercial preparations of the extract exhibit an enormous range of variation in activity; and we shall make, from our own experience, an addition to the evidence on this point. There is a pressing need for the determination of the proper dosage of the extract on a scientific basis. In the first instance it was indicated in a crudely empirical manner, and a dose which was found effective and safe *post partum* soon came to be used without discrimination, for accentuating or accelerating the pains, for example, in the earlier stages of labour. The growing record of accidents from ruptured uteri and retained placentae, has evoked a response from some manufacturers, who have put up a half-strength extract (usually corresponding to a 5 per cent. extract of fresh gland) and labelled it 'obstetrical'. Apparently the medical practitioner, when using a remedy put up in sterile solution in sealed ampoules, cannot be prevented from regarding the size of the ampoule as an indication of the dose. Nothing effective, however, has been done, or even attempted, towards a settlement of the question as to what dose is safe and effective for each particular condition. Nor is investigation of this important matter possible, under conditions in which extracts are put out under various trade names, with ostensible values varying from 1 part of fresh substance in 5, to 1 part in 20 of water, while the actual strength even of theoretically equivalent extracts varies, as we shall show, much more widely. One observer may recommend '1 c.cm. of pituitary extract' as the safe dose for a certain purpose, on the strength of experience with a '5 per cent.' extract of a certain make; another worker, accepting this advice, may give, in similar circumstances, 1 c.cm. of a '20 per cent.' extract of another make—a dose which in theory is 4 times, and in fact is quite possibly 10, 50, or even some hundreds of times as great. It is clear that no scale of dosage can be determined until some

reasonable uniformity is obtained among the preparations used. This can only be effected, at present, by physiological comparison with a standard, and there is no certain prospect of a chemical method being ever available.

II. THE CHOICE OF A PHYSIOLOGICAL METHOD.

If we could be assured that all the physiological actions of the extract, or that all those which are of therapeutic importance, were due to a single active principle, any method of determining the relative concentration of that principle, present in a sample of the extract, would be admissible. Provided that the chosen physiological reaction measured this with accuracy, it need have no more direct relation to the therapeutic effect desired than a chemical test, such as a colour-reaction, would have. The action of the extract on the melanophores of fish-scales, for example, might provide a suitable basis of measurement, as suggested by Spaeth (1918 *a*), if we knew that only one active principle was concerned in the therapeutic effects, and if this could be shown to be responsible for the whole action on the melanophores.

The position is, in fact, much less simple. The evidence available points clearly to the participation of at least two active principles, in producing even the two physiological effects of the extract which are most familiar and of the greatest therapeutic importance—the action on the blood pressure and the action on the uterus. We do not know whether the principle acting on arterial plain muscle is wholly without action on that of the uterus, or the uterine stimulant wholly free from pressor action. All that we can properly demand of any method is that it shall measure one or other of these effects with reasonable accuracy. In the case of an action such as that on melanophores, we have no proper ground for assuming that it has any relation either to the action on the uterus or to that on the arteries; it may well be due to a constituent which has no special action on either. Moreover, it is by no means easy to obtain satisfactory evidence of the identity of the constituents responsible for two different effects. In the case of the actions on the blood pressure and the uterus, we are certainly dealing with principles which are very similar as regards the conditions of their solubility and stability. Conditions such as staleness of the material, or slow heating in the making of the extract, which lead to deficiency in one kind of activity, lead to a deficiency in both. The impression is easily formed that the two types of activity always vary in a parallel manner. In an earlier communication Dale and Laidlaw (1912) stated that the correspondence was so invariable, in an experience which had already covered a very large number of samples, that it was safe to assume, for practical purposes, that only one active principle was concerned, and to use the measurement of one type of activity as an index of both. Even now it can be stated that departures from this rule are rare; but it can also be stated that they do, beyond question occur. Two extracts have been examined by one of us (H.H.D.) which were indistinguishable in their pressor action, though one of them was about four times as active as the other on

the uterus ; and Dudley's recent work (1919, 1922) has demonstrated the possibility of making at least a partial separation of the two types of activity. A very large body of evidence, showing invariable correspondence over a very wide range of samples, would, therefore, be needed before any action, other than those directly concerned in the practical uses of the extract, could safely be adopted as an index of therapeutic potency.

This being so, the choice lies between the action on the blood pressure and that on the uterus as a measure of therapeutic activity. Both measurements, indeed, ought to be made for absolute assurance of satisfactory activity in both directions. For practical purposes, however, it will probably be sufficient to adopt one or the other test, with an occasional control to ensure that no part of the process of making or sterilizing the extract is destroying one principle at a disproportionate rate.

Of the two kinds of activity available for measurement, that on the blood pressure was probably the first to be applied, though no early description is available giving details of its application. Roth (1914) compared several extracts by this method and by the uterus method, and concluded that the latter lent itself to greater accuracy. Hamilton (1916) gave an account of the test, as used by him on dogs anaesthetized with chloretone, in which he claimed to be able to detect a difference of 10 per cent. in activity. Our own experience with the blood-pressure test has been confined to experiments on the spinal cat, in continuation of those described by Dale and Laidlaw (1912). We have not observed such a delicacy of discrimination as that mentioned by Hamilton, but we have no reason to doubt its possibility in an occasional, particularly favourable, preparation. Apart, however, from the question of its accuracy, which in our experience is usually much lower than that of the test on the uterus, there is an objection to the blood-pressure test, on whatever species it is made, and under any form of anaesthetic. Howell (1898) first described the greatly reduced sensitiveness of the blood pressure to a second injection, given within an hour of the first. With small doses the phenomenon is not so pronounced, but, however small the dose chosen, the effects following the first few injections show a rapid decline, until a condition is produced in which further equal, small doses, 'given at regular intervals, cause practically equal results. At this stage the comparison of a preparation with the standard is made. Given a sufficient accuracy of comparison no objection could be raised to this procedure, if we had certain knowledge that the pressor action was entirely due to one principle. We have, in fact, no such knowledge. It is not inconceivable, or even improbable, that more than one substance may contribute to the total pressor effect. Dudley's recent work (1922) affords strong evidence in favour of this supposition, and suggests that tolerance is rapidly established for the action of the most potent of the pressor principles, leaving a remnant of more constant response to a less active principle, to which the tolerance acquired is relatively trivial. If this is the case, the blood-pressure method, in the only form in which it can afford anything like a quantitative comparison, must give an estimate, not of the total pressor activity, but of the part

of it due to a subsidiary active principle. The objection is of sufficient importance to determine choice in favour of another method, if one can be found which is equally sensitive in discrimination and free from suspicion of any kind.

The test on the isolated uterus of the guinea-pig is certainly free from this objection. Unless the successive doses are given with altogether unreasonable rapidity no tolerance is produced; on the contrary, the tendency is for the plain muscle to become gradually more sensitive in reaction as the experiment proceeds. A further and extensive experience of the method has confirmed the conclusion, drawn by Dale and Laidlaw in connexion with their first description of it, that it will detect differences of activity within much narrower limits than those which the blood-pressure method usually reveals. It has the advantage that the particular form of activity which it determines is the one which has given to the extract its widest use in therapeutics. The manipulations involved are of the simplest kind, and, since the use of the living animal is not involved, the test can be carried out in any laboratory possessing the necessary equipment, and by anybody who is endowed with the necessary patience. The method is apt to be tedious, and the fact that it calls for no special skill in manipulation makes it more so. The experimenter, during all but a small fraction of the period of some hours needed to complete an accurate comparison, is reduced to the rôle of a mere spectator; yet, if he is to get the best results which the method can yield, he must not allow his attention to be seriously diverted or his procedure to be hurried. Results cannot indeed be obtained more rapidly by the blood-pressure method, if they are to have any quantitative significance; but the more complicated and varied technique keeps the attention of the experimenter occupied, and makes the experiment less tedious.

The test on the uterus, since its first description, has been widely used, and apparently with varying success. Some workers, such as Hamilton, have dismissed it as giving merely a qualitative indication, as contrasted with the quantitative results obtained with the blood-pressure method. This is not the experience of the majority, but many have met with difficulties, and much discussion has arisen on details. Much of the more recent discussion has centred round attempts, mistaken in our opinion, to use stable chemicals to provide an arbitrary standard of activity.

More than a year of continuous work with the isolated uterus has had the effect of strengthening our conviction that, when the method is properly used, it will give results far more accurate than those obtainable by other methods. Attention to certain details, which, for the most part, are not those to which other workers have attached importance, has rendered difficulty and failure increasingly rare. Fenger (1916) states that 'often three or four guinea-pigs have to be killed before a satisfactory strip is obtained'. In our last 200 experiments we have only on some 10 occasions had to reject preparations as unsuitable.

Our conclusion, therefore, is the converse of that reached by Hamilton. We regard the test on the guinea-pig's uterus as by far the most suitable for quantitative comparisons with a standard;

but, for reasons already given, we believe that it should occasionally be supplemented by a test on the blood pressure, which, though less accurately quantitative, suffices for assurance that the pressor activity is not disproportionately defective. The choice of method being made in favour of the test on the isolated uterus of the guinea-pig, we have now to define the conditions of its performance, and the standard with which comparison is to be made.

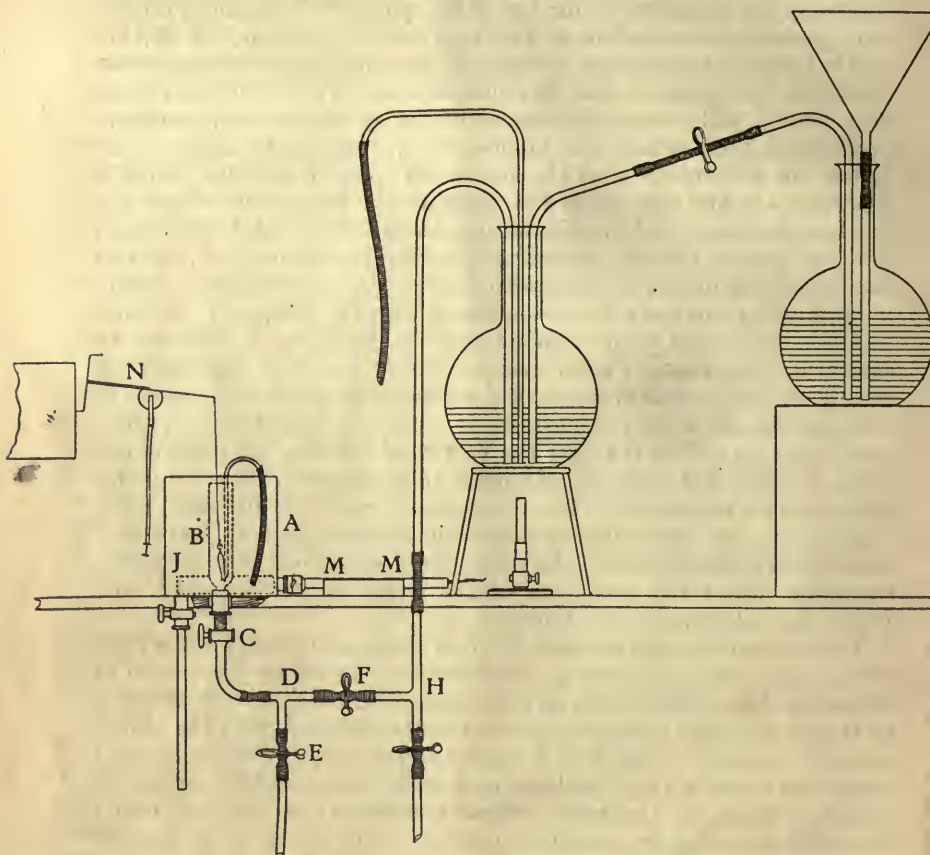


FIG. A. Sectional diagram of whole apparatus $\times 1/12$.

III. DETAILS OF THE METHOD.

(1) *The Apparatus* (Figures A and B)

The apparatus which we have used is essentially the same as that described by Dale and Laidlaw (1912), but improvements have been made in detail, which have given such increased convenience as to make a full redescription desirable. The main improvements are in the method of fixing the experimental glass chamber in the copper thermostat bath, in the method of regulating the temperature, and in the recording lever.

The thermostat bath (A) which we have in use is of stout copper,

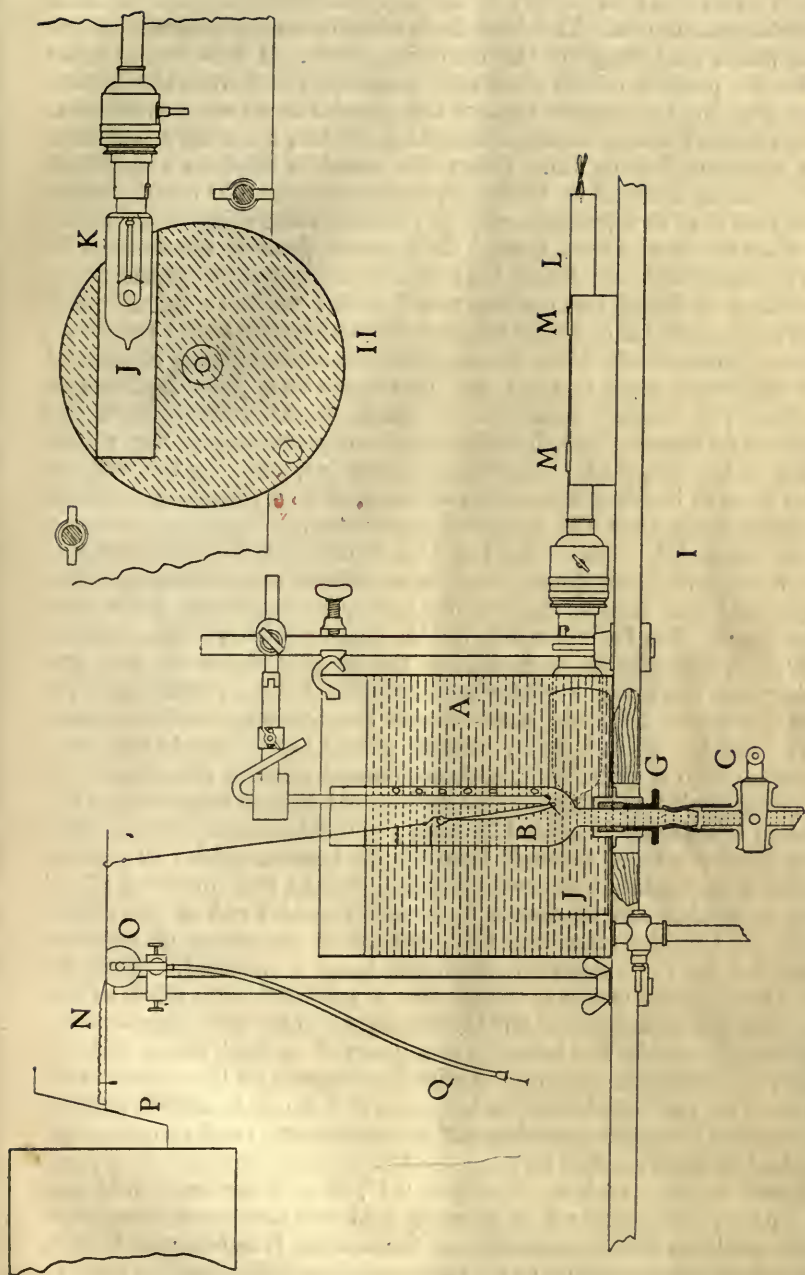


FIG. B. (I) Detailed view of thermostat bath with testing vessel showing method of attachment of uterus, writing lever, &c. $\times 1/5$.
 (II) Horizontal section of lower part of bath $\times 1/5$.

and is cylindrical in shape, being approximately 18 cm. in both diameter and depth. The bath is flat-bottomed and stands on the table which also supports the recording drum. A hole in the table allows the passage of the glass tube leading to and from the testing vessel (B). In the present form of the apparatus we use a cylindrical glass vessel of about 3.5 cm. internal diameter, i.e. with a sectional area of about 9.5 sq. cm. When the vessel is filled to a depth of 10–11 cm. its content is, therefore, approximately 100 c.cm. At its lower end it is narrowed sharply to join the connecting tube, which has a diameter of about 1 cm. This passes through the bottom of the thermostat bath, being held tightly by a packed gland (G)—a method of fixing the testing vessel which we have found much more convenient than the rubber cork indicated in Dale and Laidlaw's figure. Immediately after passing the gland the tube is connected to a wide-bore glass tap (C), and this again to a glass T-piece (D), enabling the testing vessel to be emptied and refilled from the reservoir of warm saline, by opening the tap and spring clip E or F shown in the diagram. The extra T-piece (H), enabling the system, as far as F, to be filled with warm saline prior to a change of solution, is exactly as in Dale and Laidlaw's apparatus.

The method of controlling the temperature of the thermostat is one which we have found most convenient and effective. The copper rod and Bunsen burner of the earlier apparatus have been abandoned. The bath has now a wide copper tube (J), closed at the inner end, soldered at its mouth to the wall of the bath, and projecting into the interior of the bath near the bottom, sufficiently far from the centre to avoid contact with the testing vessel. A small blind tunnel is thus formed, passing nearly across the bath. This accommodates a cylindrical, carbon-filament electric glow-lamp (K). The lamp socket is attached to a cylindrical hollow brass holder (L), through which the insulated flex passes to the lamp socket. This brass holder passes through two simple friction-guides (M) which are fixed to the table, and which fix the height and direction of the lamp so that it can be drawn by a touch in and out of the tunnel in the side of the tank, the temperature of the water in which is controlled by the extent to which the lamp is inserted. With the laboratory at its ordinary temperature and the bath filled in the first instance with water at 37° C., the lamp will keep the temperature remarkably steady for hours, when inserted so that about half its length lies within the tunnel. A slight withdrawal if the temperature tends to rise, a slightly deeper insertion if it tends to fall, is all that is necessary; careful watching of the thermometer, and an occasional small adjustment suffice to keep the temperature within 0.5° C., and it would be easy to keep it within 0.1°, if such accuracy had any advantage. The control is so easy that we have not thought it worth while to devise a mechanism for making it automatic, though this should not be difficult if a thermostat on this principle were to be used for observations needing a more accurate regulation of temperature. The principle is not at all new; it was used by Professor A. N. Richards when working with one of us in 1917, and is doubtless familiar to many workers; but this particular method of applying it has proved so convenient for this special

purpose that it seems worth while to describe it in detail for the benefit of others.

The testing vessel bears graduation marks corresponding to contents of 100 and 125 c.cm., measured from the closed tap c. Before each experiment the thermostat bath is filled with water at 37° C. up to the mark on the testing vessel corresponding to the volume of 125 c.cm. measured from the tap. At each change of the fluid the level of the water gives a useful guide to approximate adjustment, and final adjustment is made to the 125 c.cm. mark.

The lever (N) which we have used recently differs from that given in the earlier description. The fulcrum pin, with conical end-bearings, passes through the centre of a small circular slab of ebonite (o). Rather less than the upper half of the circle is cut away, leaving a flat surface to which the lever is attached. The lever is made of a slip of thin magnalium, folded so as to have an L-section, which gives it rigidity in both horizontal and vertical planes. The horizontal portion is attached near its middle to the flat surface of the ebonite sector and is pierced with a series of holes. The vertical portion is cut by a series of corresponding grooves. The holes and grooves enable the hook terminating the thread from the uterus to be attached, or small weights to be hung, at any desired distance from the fulcrum. One end of the lever projects over the mouth of the testing vessel, and the other carries a delicate frontal writing-point (p) of the type described by our colleague Lovatt Evans (1919). The frontal writing eliminates the difficulty of using an ordinary cylindrical recording drum for the long curves drawn by the contracting uterus, and the absence of circular distortion enables the comparison of the heights of successive curves to be made with much greater accuracy. With the adoption of the frontal lever the ink-writing point, formerly used, has been abandoned in favour of the glass point writing on a delicately smoked surface. As in the earlier pattern a small brake is used for fixing the lever while the fluid is changed. This consists, as before, of a small Bowden cable (q), made for the shutter-release of a photographic camera, on pressing which a small steel rod impinges radially on the under, circular surface of the ebonite segment to which the lever is attached, and so fixes the lever.

The lever which we have actually in use has a total length of 19 cm. The distances from the fulcrum to the hinge of the frontal writing point and to the point of attachment of the thread from the uterus are, respectively, 10.5 and 8.5 cm.; so that the records of contraction are made with a magnification of only about 5:4. The whole horn of the uterus, which we always use, gives contractions of such length as to require no magnification; indeed, any greater magnification would bring the summits of the curves beyond the range of reasonable accuracy of the frontal lever. We adjust the weighting according to the size or strength of the horn being used, a small weight being hung in notches of the lever at different distances from the fulcrum. It is impossible to give a general indication of the effective load required. It is necessary to load each preparation to such an extent that, when unstimulated, it relaxes to the full extent of its length, but not so heavily as to obliterate, or seriously impede

the small, automatic rhythm which the relaxed uterus normally shows. The average effective load (i.e. the appropriate load for application at the same distance from the fulcrum as the attachment of the thread from the uterus) is about 0.5 gm.; but as little as 0.2 gm. will in some cases suffice, and more than 0.5 gm. may be needed.

(2) *The Locke-Ringer Solution.*

For some years past we have used a saline solution of the same composition for this purpose. Its formula was gradually established by deliberate trial, assisted by accident, as the most suitable. It differs on two small points from the Locke solution in most general use for isolated mammalian hearts, &c. (1) The proportion of sodium bicarbonate is 0.05 per cent., in place of the more usual 0.015 per cent. This proportion of the bicarbonate was indicated by Dale and Laidlaw in their description of the method in 1913, but special attention was not drawn to it. We know of one case in which other workers have fallen into difficulty with the method through use of a Ringer solution containing only 0.015 per cent. of the bicarbonate. (2) The other difference from an ordinary Ringer's solution which we have found advantageous, and which was adopted after the publication of Dale and Laidlaw's paper, is the addition of a minute proportion of magnesium chloride. Magnesium chloride as a constituent of a Ringer solution was first used by Tyrode, in the now familiar fluid, in which segments of rabbits' intestine preserve such a regular rhythm and remain so long excitable. We tried Tyrode's solution for the uterus many years ago, and found that it depressed the excitability to the pituitary extract to a degree which was inconvenient. We found, however, that the introduction of a smaller proportion of magnesium into the solution we had previously used had a favourable effect in weakening the tendency to irregular spontaneous rhythm and preserving the excitability of the plain muscle to pituitary extract. Kochmann (1921) has recently recommended, as facilitating the testing of pituitary extracts on the uterus, reduction of the calcium chloride till the spontaneous rhythm is completely suppressed. Dale and Laidlaw pointed out in 1912 that reduction of the calcium to one-half the normal strength—a much smaller reduction than that advocated by Kochmann—produced a condition in which the responses to successive equal doses of the pituitary extract showed a rapid decline. We regard any method which involves the suppression of automatic rhythm by reduction of calcium as quite unsuitable for our purpose.

The formula of the Ringer's solution which we have now used for some years is as follows:

NaCl	.	.	.	.	.	0.9 gm.	/
KCl	.	.	.	.	.	0.042	
CaCl ₂	.	.	.	.	.	0.024	
NaHCO ₃	.	.	.	.	.	0.05	
MgCl ₂	.	.	.	.	.	0.0005	
Dextrose	.	.	.	.	.	0.05	
Water	.	.	.	.	.	100 c.cm.	

It is possible that others may find slight changes in the formula advantageous. The only point on which we wish to insist is the desirability of stating the composition of the Ringer's solution used, when comparative results are presented. This is particularly important when an artificial standard of comparison is used. Changes in the composition of the Ringer's solution modify the sensitiveness of the plain muscle to the pituitary extract; they also modify its sensitiveness to substances like histamine and potassium chloride, but not necessarily to the same extent, or even in the same direction. We shall return to this point in considering the choice of a standard.

(3) *The Choice of a Guinea-Pig.*

There has been some controversy as to the most suitable size of the guinea-pig furnishing the uterus for this test. Dale and Laidlaw recommended that young virgins of not more than 350 gm. weight should be used. Roth (1914) stated that they should preferably weigh from 275 to 350 gm., while other workers have obtained better results with younger animals weighing from 175 to 250 gm. When the strength of pituitary extract is indicated in terms of an artificial standard such as histamine, it may be of importance to prescribe the age of the guinea-pigs to be used by indication of a limited range of body weight. It is quite possible that the uteri from guinea-pigs of 200 gm. may show a different average ratio of sensitiveness to pituitary extract and histamine from that exhibited by uteri from older guinea-pigs of, say, 350 gm. For the method as we use it, however, any guinea-pig's uterus is suitable, provided only that it shows a delicacy of grading in its responses to different doses of the same pituitary preparation, constancy in the height of contraction by which it responds to the same sub-maximal dose, and freedom from hampering automatic activity. We most commonly obtain such uteri from guinea-pigs weighing between 200 and 300 gm. In smaller guinea-pigs the uterus is usually too delicate and inert; in larger ones it is apt to be too sensitive, and prone to automatic contractions; but we have often obtained useful preparations from animals outside these limits of weight. There is a matter more important than age or weight, to which we have not seen definite reference in the writings of other workers. It is agreed that the uterus from the parous guinea-pig is too excitable and irregular in activity, and virgins are always chosen, but it does not seem to be generally recognized that the uterus of the virgin on 'heat' is unsuitable. The condition is easily recognized in life by the swollen and moist condition of the vulva, and, when such an animal is killed, the state of the uterus is also easily detected. Whereas, in the condition of sexual rest, the uterus is found to be slender, pale and almost fully extended, the oestral organ is short, thick and vascular, being so contracted that the fallopian tube is extended and almost straight. The horn of such a uterus will often relax rapidly in Ringer's solution, but it is excitable, and prone to acquire a large, irregular, automatic rhythm. At certain times of the year the majority of young females taken from a mixed pen are found to be in this condition, which renders

the uterus unsuitable for the accurate conduct of the test. This is, no doubt, the cause of the difficulty which some workers have experienced in finding a suitable preparation; it has at times, in the past, almost driven us to the conclusion that the test could be properly carried out only at certain times of the year. The difficulty was one which obviously had to be eliminated, if the test was to have general utility; and by a simple expedient we have eliminated it almost completely from our practice. If the young females are separated at an early age, and kept in pens or cages quite separate from the males, it is, in our experience, very unusual to find one in which the uterus is unsuitable, on account of oestral activity, until they have attained a weight well over 300 gm.

Our recommendations as regards method of choosing guinea-pigs are, therefore, as follows: the young females should be segregated, as soon as they are ready to leave the mother; they should be used, preferably, when between 200 and 300 gm. in weight; and the absence of oestral activity should be verified by external inspection, before the animal is killed.

(4) *The Preparation of the Uterus.*

We have never found reason for departing from Dale and Laidlaw's original recommendation to use the whole horn of a uterus, suspended between a cervical tag at one end and the ovary at the other. Workers in the Washington Laboratory have been inclined to insist on the use of a portion of the horn 'measuring from 1 to 2 centimetres in length' for the official test, and to attribute the varying results obtained by other workers, partly to the choice of greater portions of the uterus, with or without ovaries. There may be some unexplained need for this rigidity of prescription if the strength of the pituitary extract is to be laid down in terms of equivalence to an artificial standard; though we must admit some personal doubt as to the exact significance of '1 to 2 centimetres', seeing that the same horn may show a physiological variation in total length from 1 to 5 centimetres or more. A prescription of the middle, upper, or lower half, third or quarter of the horn, whichever is intended, would, we think, be clearer. But, whatever be the exact intention, we can see no objection to the use of any special part of the organ by a worker who finds that he can obtain with it clearer and more regular results than with the whole. For our own part, having tried the preparation with the ovary and fallopian tube removed, we are definitely of opinion that we obtain a finer delicacy of discrimination, between nearly equal doses, when it is retained. With the range of dosage in which we find that the finest differences are detected, i.e. with doses about 60 to 80 per cent. of that producing a maximal effect, the curve is broken near its summit by a series of small contractions and relaxations, when the ovary forms one of the points of suspension. We find that similarity in the depth of this indentation of the summit is an additional point of some value in enabling us to judge of the exact equivalence of two doses. With a smaller dose, not only is the absolute maximum lower, but the indentation is deeper; with a larger dose, the indentation becomes nearly extinguished.

The retention of the ovary seems to us to have only one possible disadvantage. If the preparation needs more than the average weighting to secure proper relaxation and a duly small rhythm, there is a tendency for the upper delicate portion to undergo gradual stretching, with a consequent lowering of the level of full relaxation, as the experiment proceeds. A continuously falling base line makes accurate comparison more difficult. In such cases we have found it useful, when once full relaxation has been obtained, to introduce a small stop which limits the fall of the lever to this point. Each curve then starts from exactly the same level, and we have obtained some very accurate and concordant determinations under such conditions.

(5) *Method of Carrying out the Test.*

It will be evident, from the description of our apparatus given above, that we have adhered to the method of making the final dilution of the extract in the actual testing vessel. The material for test is prediluted, usually from 100 to 200 times, with Ringer's solution, so that the dose to be added to the bath shall not be too small for reasonable accuracy of measurement, or so large that its addition, unwarmed, will sensibly affect the temperature of the solution in the testing vessel. We aim at working with a dose of 0.3–0.6 c.cm. We are aware that a more perfect accuracy of dilution can be attained by the method adopted by Roth (1914) and others, of making the required dilutions in bulk, and placing them in aspirators in the same large warming-tank as the testing vessel, so that, in changing from pure Ringer's solution to the dilution of extract, no measurement of volume is involved. This method, however, is obviously much more cumbrous. Whenever a small change of dosage is required, a new dilution must be made on a large scale, and time given for it to come to the required temperature. If we correctly interpret the description, it would appear, moreover, that the bath must be emptied of Ringer's solution and refilled with the dilution whenever a dose is given. If this is the case, we should expect the stimulus of exposure to form an undesirable complication to the effect of the dilution of extract. In any case, we are convinced that the method of making the dilution by adding a small measured volume directly to the testing vessel, though less ideally accurate, involves no such error as the method itself is capable of detecting. Successive equal doses of the same preparation produce indistinguishable results; indeed, if they do not, we know that we are dealing with a uterus which is unsuitable for the test, and discard it—a necessity which is fortunately rare in our experience. Our method, on the other hand, gives a freedom and elasticity in the choice and change of dose, which we regard as essential to the test as we carry it out. Further details of procedure can more suitably be given when we have discussed the choice of a standard.

IV. SUGGESTED ARTIFICIAL STANDARDS.

The determination of the strength of the extract is necessarily comparative. A rough idea of the activity could be obtained, no doubt, by finding the weakest dilution which would suffice to produce

contraction to the maximum. It is admitted, however, by all who have used the method, that different preparations of the uterus exhibit wide differences in sensitiveness, so that the only satisfactory basis, for accurate measurement of activity, is a determination of the dilution which produces an effect identical with that produced by a standard preparation.

There would obviously be great advantages in the use of a stable standard of known chemical constitution, which could be prepared perfectly pure, and weighed out exactly. In the course of their early work with the test Dale and Laidlaw made a few attempts to use substances of this kind as a standard. Among others they tried histamine, on the action of which they had, at the time, been recently working. They observed what appeared to be obvious differences in the relative sensitiveness of different uteri to histamine and to the pituitary extract. One preparation would show a high degree of sensitiveness to histamine and a low degree to the pituitary extract, and in another the reverse relation seemed to hold. They accordingly suggested, as the only immediate possibility, the use, as an empirical standard, of a simple extract of the fresh substance of the infundibular pituitary lobes of oxen, sterilized in ampoules, and renewed at frequent intervals.

The suggestion was admittedly not ideal in every way, and several attempts have been made to replace it by the use of pure chemical standards. Roth (1914) worked out with great care a method in which histamine was used as the standard, and this method was subsequently prescribed officially in the U.S.P. IX. Several workers published criticisms of the method, which was found difficult to use. It has further been suggested that the strength of pituitary extract, officially indicated in terms of equivalence to histamine, is much below that of a good preparation.

Trendelenburg and Borgmann (1920), apparently without knowledge of Roth's publications, also suggested the use of histamine as a standard.

A later publication, by Spaeth (1918 b), from the same laboratory as that of Roth (the Hygienic Laboratory of the Public Health Department, Washington), seems to withdraw the suggestion of histamine as a suitable standard, not on account of the difficulties which formed the main ground of external criticism, but because further investigation had produced evidence that histamine was not indefinitely stable, either in sterilized solution, or even as the dry hydrochloride.

We may say at once that, as the result of some years of continuous experience, we have observed nothing to give any support to this alleged instability of histamine. We have been accustomed to use the diphosphate, which forms large, well-formed crystals, free from water of crystallization and not hygroscopic. We have for years used solutions prepared from the same sample, and have also had experience of numerous samples, without observing anything which could create even a suspicion of deterioration, or of variation in the activity of this pure salt. We know, on the other hand, that solutions, unless sterilized with great care, readily support the growth of bacteria and moulds, with a consequent rapid loss of activity. We can only suppose that the samples of the hydrochloride, available to

American workers, were incompletely dry, so that decomposition by micro-organisms became possible; or that some unknown impurity in some way affected the stability of the salt or its solutions.

Histamine, as a standard of reference for the activity of pituitary extracts cannot therefore, in our opinion, be rejected on the ground of its supposed instability. Spaeth, however, for this reason suggested its replacement by potassium chloride, which is beyond question stable, and which, in relatively high concentrations, produces contraction of the uterine muscle which, superficially, are not wholly dissimilar from those produced by the pituitary extract. We considered it desirable, therefore, to give both of these suggested standards a thorough trial.

In forming a conclusion as to the suitability of a substance, other than the pituitary extract itself, to serve as a standard of activity for this extract, there are two points to be kept clearly in mind.

1. Preparations of the isolated uterus from different guinea-pigs show, under conditions otherwise identical, a rather wide variation of sensitiveness to the pituitary extract. It is, indeed, the existence of such variation which creates the need for a standard of reference. It is obvious that the use as a standard of some other substance will be completely fallacious, unless the sensitiveness of the uterine muscle to this standard substance shows a strictly parallel variation.

2. During the course of an experiment the uterine muscle usually exhibits a progressive increase of excitability to the pituitary extract. Since each test involves the trial of a series of doses, until equivalent doses of the standard and of the preparation being tested are discovered, it is evident that an accurate final result can only be obtained if any change in excitability to the pituitary extract, occurring during the course of an experiment, is accompanied by an exactly parallel change in excitability to the standard substance.

We accordingly carried out a series of experiments to determine whether histamine or potassium chloride was suitable, in these respects, for use as a stable standard of activity.

(1) *Experiments with Histamine.*

The salt used was histamine diphosphate— $C_5H_9N_3(H_3PO_4)_2$. The molecular weight is 307, while that of histamine base is 111; so that 10 parts of base are contained in 27.7 of the salt. It is our custom, for convenience of comparison with results obtained with other salts of histamine, to make up our solutions and express dosage in terms of the free base. 28 mgm. of the diphosphate dissolved in 1 litre of Ringer's solution gives, with sufficient approximation to accuracy, a solution containing 0.01 mgm. per c.cm. Addition of 0.5 c.cm. of such a solution to a testing vessel containing 125 c.cm. gives a final dilution of one part of histamine base in 25 millions. (The 'standard' dilution suggested by Roth, and adopted by the U.S.P. IX, was 1 part of histamine hydrochloride in 20 millions, which is equivalent to 1 part of the base in 26.5 millions.) We prepared a fresh quantity of the 1 in 100,000 histamine solution on each day on which the test was carried out. The weighing and

measurement were done accurately, and variation in the strength of the solutions so prepared can be excluded.

The pituitary extract, which formed the test preparation for standardization, was prepared from desiccated posterior-lobe substance, the method used being similar to that described later. The extract was so made that 100 c.cm. represented 0.05 gm. of the dry substance, and a sufficient quantity for the whole series of tests was prepared in one operation. Immediately after filtration, and while still hot, the extract was distributed into clean test-tubes. These were plugged with cotton-wool and immersed for 5 minutes in a common boiling water-bath. They were then stored in the refrigerator, at a temperature just below 0°C, till required. For each

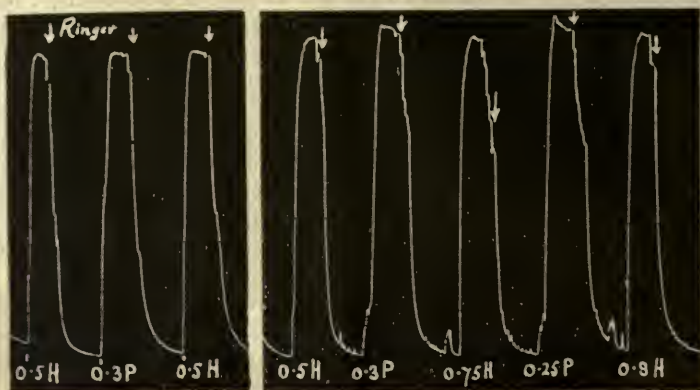


FIG. 1. Comparison of pituitary extract, P (0.05 per cent.), with histamine solution, H, 1 in 100,000. Change of Ringer at arrow. Note equivalence of 0.5 c.cm. H and 0.3 c.cm. P in first part of tracing, and the inferiority of 0.8 c.cm. H to 0.25 c.cm. P in later part.

of the tests a fresh tube of the extract was drawn from the cold store. In the following records 'H' represents the solution of histamine, 1 in 100,000, and 'P' represents the pituitary extract, 0.05 per cent. The equivalents recorded do not represent all the doses administered, but the approximations obtained at different stages of the experiment. In some cases a rough equivalence was obtained quite early. In these cases further doses were administered to discover whether equality was maintained. In other cases the earlier doses merely indicated certain limits, and, with these as guide further doses were tried, with a view to obtaining an exact match between those limits.

In all the records, as reduced for reproduction, 1.5 mm. represents approximately 1 minute, when the drum is in motion.

(a) Guinea-pig weighed 250 gm. The results at different stages of the experiment were as follows:

1. 0.5 c.cm. H = 0.3 c.cm. P. (See Fig. 1.)
2. 0.5 c.cm. H < 0.3 c.cm. P.
3. 0.75 c.cm. H < 0.3 c.cm. P.
4. 0.75 c.cm. H < 0.25 c.cm. P. (See Fig. 1.)
5. 0.8 c.cm. H < 0.2 c.cm. P.

(b) Guinea-pig weighed 250 gm.

1. 0.5 c.cm. H < 0.4 c.cm. P.
2. 0.5 c.cm. H > 0.3 c.cm. P.
3. 0.5 c.cm. H = 0.35 c.cm. P.

(c) Guinea-pig weighed 250 gm.

1. 0.5 c.cm. H < 0.5 c.cm. P.
2. 0.5 c.cm. H = 0.4 c.cm. P.
3. 0.5 c.cm. H = 0.35 c.cm. P. }
4. 0.8 c.cm. H = 0.2 c.cm. P. } (See Fig. 2.)

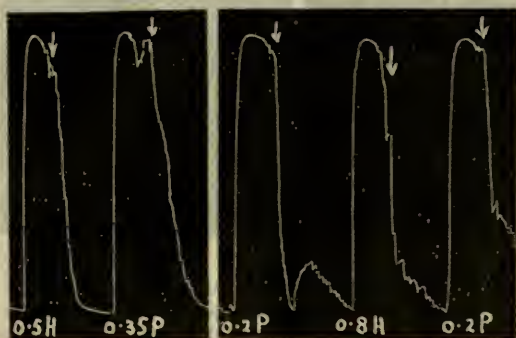


FIG. 2. Determination on another uterus.

Comparison of same pituitary extract (P) and histamine solution (H) as in Fig. 1.

Note equivalence at early stage of 0.5 c.cm. H and 0.35 c.cm. P, and at a slightly later stage of 0.8 c.cm. H and 0.2 c.cm. P.

(d)

1. 0.5 c.cm. H = 0.5 c.cm. P. (See Fig. 3.)
2. 0.5 c.cm. H < 0.4 c.cm. P.
3. 0.5 c.cm. H < 0.3 c.cm. P.)
4. 0.5 c.cm. H > 0.25 c.cm. P.) At this stage 0.5 c.cm. H =
5. 0.5 c.cm. H < 0.25 c.cm. P.) (approx.) 0.275 c.cm. P.
6. 0.7 c.cm. H = 0.25 c.cm. P.
7. 0.75 c.cm. H > 0.25 c.cm. P.
8. 0.7 c.cm. H > 0.3 c.cm. P. }
9. 0.6 c.cm. H < 0.3 c.cm. P. } (See Fig. 3.)

(e) Guinea-pig weighed 210 gm.

1. 0.5 c.cm. H > 0.3 c.cm. P. (See Fig. 4.)
2. 0.5 c.cm. H > 0.4 c.cm. P. }
3. 0.5 c.cm. H < 0.5 c.cm. P. } At this stage 0.5 c.cm. H =
4. 0.5 c.cm. H < 0.4 c.cm. P. } = (approx.) 0.45 c.cm. P.
5. 0.75 c.cm. H < 0.4 c.cm. P.
6. 0.75 c.cm. H < 0.3 c.cm. P. (See Fig. 4.)

A superficial inspection of these results is sufficient to show that histamine does not fulfil either of the conditions, above laid down, for a suitable standard for the pituitary extract.

If we restrict our attention, in the first instance, to the earlier stages of each experiment, and accept as most correct the first equation of activities obtainable, we obtain, in the series of five experiments, the following equivalents of the pituitary extract for 0.5 c.cm. of the histamine solution :

(a) 0.3, (b) 0.35, (c) 0.4, (d) 0.5, (e) 0.45 c.cm.

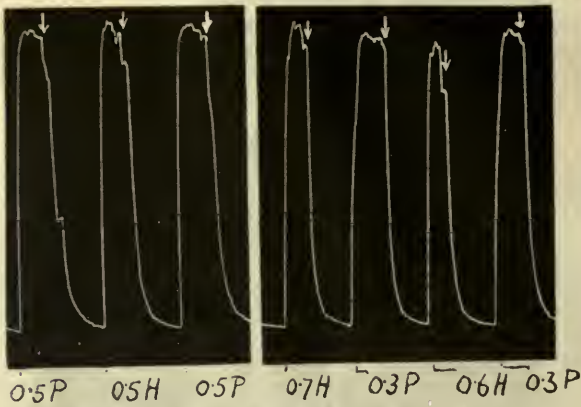


FIG. 3. Determination on another uterine horn.

Same solutions of pituitary extract and histamine as previous figures. There was first an equivalence between 0.5 c.cm. P and 0.5 c.cm. H, while later 0.3 c.cm. was less than 0.7 c.cm. H, but greater than 0.6 c.cm. H.

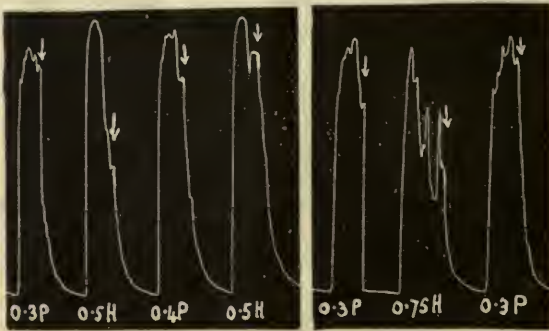


FIG. 4. Same solutions of pituitary extract and histamine as in Figs. 1-3. At first 0.5 c.cm. H is greater than 0.4 c.cm. P, while later 0.75 c.cm. H is less than 0.3 c.cm. P.

Experiment (d) gives us an equivalence practically the same as that demanded by the U.S.P. IX. This stipulates for a 10 per cent. extract of fresh gland substance, which, in a dilution of 1 in 10,000, shall match a solution of histamine hydrochloride 1 in 20,000,000. We may take the fresh gland substance as containing about 20 per cent. of dry matter, so that a 10 per cent. extract of fresh gland is equivalent to a 2 per cent. (1 in 50) extract of dry gland substance. This diluted 10,000 times gives a fluid containing the extract from 1 part of dry gland in 500,000. We start with a dry gland extract diluted to 0.05 per cent., i.e. to represent 1 part of dry gland in 2,000. We add 0.5 c.cm. of

this to 125 c.cm., thus further diluting 250 times, and making the final dilution 1 in 500,000, which, in this experiment, is initially equivalent to 1 part in 25 millions of histamine base, which, as we have seen, is practically the same as 1 in 20 millions of histamine hydrochloride.

We may conclude, then, that an equivalence such as that found in the initial stages of (d) would entitle the manufacturer to pass a batch of extract as of standard strength according to U.S.P. IX. If, however, the test at his disposal was similar to (a), in which 0.5 c.cm. of the histamine solution was initially matched by 0.3 c.cm. of the pituitary dilution, he would be required to add 2 parts of water to every 3 of the extract before issuing it; and (b), (c), and (e) would entitle him to make smaller, but still important dilutions. We may add that we have not made an effort to find examples of extreme discrepancy; on the contrary, we gave the proposed standard every chance to justify itself, by conducting the whole of the series of experiments within a few weeks, on as uniform a batch of guinea-pigs as we could collect. We have no doubt that, by throwing our net wider, we could have produced some far more striking discrepancies. But, with the greatest pains to secure uniformity of material and conditions, and basing our estimates on the earliest match obtainable in each experiment, we still find such wide discrepancies between individual determinations made on the same material, that we are forced to the conclusion that *the uteri from different guinea-pigs, even under the best conditions, vary independently in their sensitiveness to histamine, and to pituitary extract respectively, and that these variations are such that the use of histamine as a standard for pituitary extract is unsatisfactory, on this ground alone.*

When we come to consider the second condition, which we indicated as necessary for the satisfactory use of an artificial standard, viz. that in any one experiment the sensitiveness of the uterus to the standard should show a change parallel to its change (usually an increase) of sensitiveness to the pituitary extract, it is at once evident that histamine fails even more conspicuously to fulfil our demand. In every experiment in which the comparison was continued for a long time, i. e., in all except (b), there is obviously a progressive increase in sensitiveness to the pituitary extract, and a progressive decrease in sensitiveness to histamine. The extent of the change involved can be appreciated from inspection of Figs. (1), (2), (3), and (4). Reference to Experiment (a) (Fig. (1)) will be sufficient. Here we have, initially, 0.5 c.cm. H = 0.3 c.cm. P. As we have indicated above, on the U.S.P. standard, the manufacturer should, on this finding, dilute 3 parts to 5 before issue. Finally, however, 0.8 c.cm. H. fails to produce as big an effect as 0.2 c.cm. P, so that, if this be accepted as the true equivalence, a more than fourfold dilution is required. There seems, indeed, a tendency to greater regularity in the finding of this final 4:1 ratio, than in the initial match. It might be suggested that this later evaluation is the true one; it is certainly obtained at a stage of the experiment at which, in comparing one pituitary extract with another, we expect to obtain the most accurate discrimination and the most definite equivalence. The suggestion, however, cannot be accepted. The extract with which we were working in this experiment had, in terms of dry gland

substance, what we regard as a very low level of activity. The dry material from which it was made had been kept in the laboratory for about two years; and, though it had apparently attained a relative stability, and yielded very uniformly active extracts, it had lost approximately three-quarters of the activity of the freshly dried infundibular substance. The fact that the lower initial value we obtained for the activity of the extract, in comparison with histamine, would make it of standard activity, according to the U.S.P. IX, strongly confirms the suggestion, made from several quarters, that the standard was, in any case, much too low. To accept a determination which would make this weak extract four times as strong as the U.S.P. standard would be to reduce the latter to an absurdity. Nor would a new definition enable these late values to be accepted. There is no finality in the whole procedure. With the sensitiveness of the uterus to the two preparations constantly changing, and moving, on the whole, in divergent directions in the two cases, it is possible to get almost any value, according to the length of the experiment, and the stage at which an approximate match is accepted as giving the correct result. Clearly then, *on the further ground that, in the course of an experiment, the sensitiveness of the uterus to histamine and to pituitary extract changes in opposite directions, histamine is unsuitable for use as a standard.*

(2) Experiments with Potassium Chloride.

We tested Spaeth's suggestion (1918 b) to use potassium chloride as a standard, in a series of experiments made on similar lines to those on the use of histamine. The extract of pituitary substance was made by an identical procedure from the same sample of dried gland, and again a supply was made sufficient for the whole series, distributed into tubes, sterilized, and stored below 0° C. The potassium chloride solution was made up to a concentration of 19.68 per cent. According to Spaeth's indication, 0.5 c.cm. of this solution, added to 100 c.cm. of Ringer's solution, raising the concentration from 0.042 per cent. to 0.14 per cent., should produce a contraction of the uterus suitable for comparative testing. Our testing vessel held 125 c.cm., and in most cases a smaller dose of the KCl solution than 0.5 c.cm. sufficed to produce a nearly maximal effect. Four complete tests were carried out. In the records K represents the strong KCl solution, P, as before, pituitary extract diluted to represent 0.05 per cent. of dry gland substance.

(a) Guinea-pig weighed 275 gr.

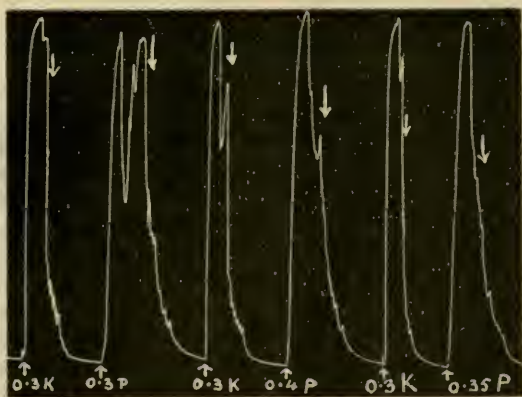
0.2 c.cm. K < 0.4 c.cm. P.	} (See Fig. 5.)
0.3 c.cm. K > 0.3 c.cm. P.	
0.3 c.cm. K < 0.4 c.cm. P.	
0.3 c.cm. K = 0.35 c.cm. P.	

(Interval of 30 minutes.)

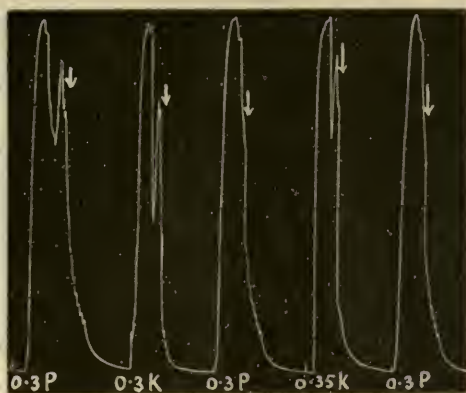
0.3 c.cm. K < 0.35 c.cm. P.	} (See Fig. 5.)
0.3 c.cm. K < 0.3 c.cm. P.	
0.35 c.cm. K = 0.3 c.cm. P.	

Later.

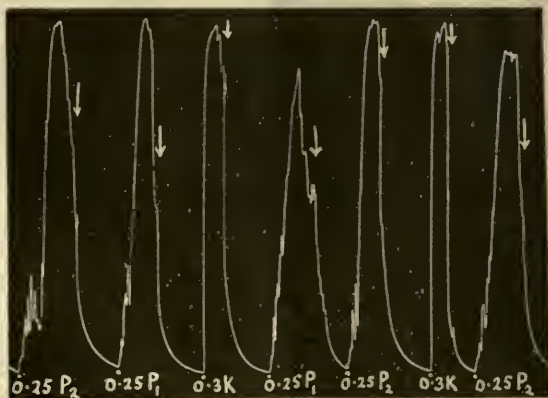
0.3 c.cm. K < 0.25 c.cm. P.



(a)



(b)



(c)

FIG. 5. Comparison of the same pituitary extract (P) (strength 0.05 per cent.) as used in Figs. 1-4, with KCl solution (K) of strength 19.68 per cent.

In section (a) an equivalence between 0.3 c.cm. K and 0.35 c.cm. P is seen. In section (b) 0.35 c.cm. K is equivalent to 0.3 c.cm. P. In section (c) is seen a curious effect apparently following the action of KCl on the isolated uterus. The first two doses in section (c) are from different tubes of the same pituitary extract. They are equivalent in action. Following a dose of 0.3 c.cm. K, another dose of the pituitary extract is much less in its effect. See text.

In this experiment, in its later stages, the opportunity was taken to compare two tubes of the pituitary extract, with the result that an exact equivalence of effect was obtained with successive doses; $0.3 P_1 = 0.3 P_2$, and $0.25 P_1 = 0.25 P_2$. At this stage a dose of $0.3 K$, following two doses of $0.25 P_1$, gave a definitely smaller effect than these; it was found, however, that another dose of $0.25 P$, following $0.3 K$, gave a subnormal effect, while with a further dose of P the reaction returned to the former level (see Fig. 5, section *c*). This apparent depression of the reaction to a submaximal dose of pituitary extract, by an immediately preceding reaction to KCl , was observed again on repeating the sequence; and it occurred at a stage when the uterus was responding to successive, equal doses of pituitary extract with mechanical regularity.

(b) Guinea-pig weighed 250 gm.

Irregular results were obtained at first, but, when response became fairly uniform, the following comparisons were obtained:

0.4 c.cm. K	>	0.6 c.cm. P.	} (See Fig. 6.)
0.3 c.cm. K	=	0.6 c.cm. P.	
0.4 c.cm. K	<	0.6 c.cm. P.	
0.4 c.cm. K	<	0.5 c.cm. P.	
0.4 c.cm. K	>	0.5 c.cm. P.	
0.4 c.cm. K	>	0.6 c.cm. P.	
0.4 c.cm. K	=	0.7 c.cm. P.	

(c) Guinea-pig weighed 300 gm.

0.3 c.cm. K	<	0.3 c.cm. P.
0.3 c.cm. K	<	0.25 c.cm. P.
0.4 c.cm. K	<	0.3 c.cm. P.
0.4 c.cm. K	=	0.25 c.cm. P.
0.4 c.cm. K	=	0.25 c.cm. P.
0.3 c.cm. K	<	0.25 c.cm. P.

(d) Guinea-pig weighed 250 gm.

0.5 c.cm. K	<	0.5 c.cm. P.
0.6 c.cm. K	=	0.5 c.cm. P.
0.6 c.cm. K	<	0.4 c.cm. P.
0.7 c.cm. K	>	0.4 c.cm. P.
0.6 c.cm. K	=	0.4 c.cm. P.

(See Fig. 7.)

The results show both the defects which were observed in the attempt to use histamine as a standard. Different ratios between the activities of KCl and of the pituitary extract are obtained in the different experiments, and the same ratio does not hold throughout the course of a single experiment. In the latter respect the results with the potassium standard are less disconcerting than those with histamine. Variation in the course of an experiment is observed; for example, in experiment (*a*) we find, initially, a good match between $0.3 K$ and $0.35 P$, and later between $0.35 K$ and $0.3 P$. The ratio has become inverted, but the discrepancy is not so glaring as in some of the histamine tests. In experiment (*c*) the ratio seems to remain unchanged. When change occurs it seems rather to take the form of a capricious variation than that of the progressive divergence seen

with histamine. The fact that a dose of pituitary extract may have its effect modified by an immediately precedent response to KCl possibly accounts for some of the anomalies. When the results of different experiments are compared, on the other hand, the discrepancies obtained with KCl as standard are at least as great as those with histamine. If we take the records of the above series and calculate, from the first match obtained in each experiment, the volume of P apparently equivalent to 0.5 c.cm. K, we obtain the following results: (a) 0.58 c.cm., (b) 1.0 c.cm., (c) 0.31 c.cm., (d) 0.41 c.cm. Spaeth's suggestion is that the effect of a commercial extract diluted to 1 in 4,000 should be equal to that of his standard potassium dilution, which he fixes at 0.14 per cent. This dilution would be produced by the addition of 0.5 c.cm. K in our experiment. The dilution of an ordinary extract, representing 10 per cent. of

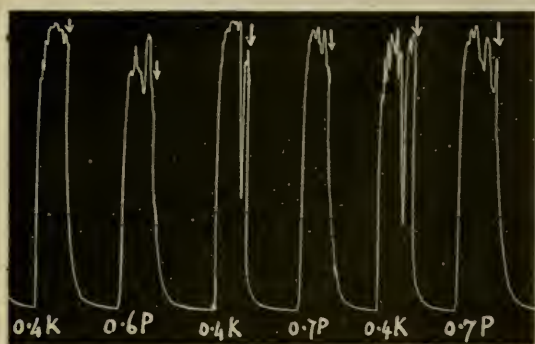


FIG. 6. Comparison of pituitary extract (P) and KCl solution (K) of the same strengths as in Fig. 5, on a different uterus. Equivalence between 0.4 c.cm. K and 0.7 c.cm. P.

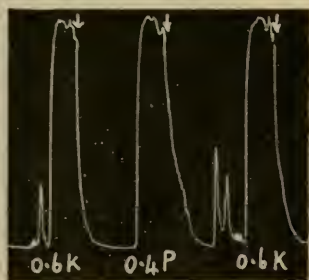


FIG. 7. Comparison of same pituitary extract (P) and KCl solution (K) as in Figs. 5 and 6. Equivalence between 0.6 c.cm. K and 0.4 c.cm. P.

fresh gland substance, to the extent of 1 in 4,000 would be equivalent to 1 part of dry gland substance in 200,000, as shown above. To obtain this dilution with our extract we should have to add 1.25 c.cm. P. So that, on the lowest estimate of its activity (b), it is too strong, and needs dilution in the proportion of 100 to 125. On the estimate given by experiment (c) it is many times too strong, and needs dilution in the proportion of 31 to 125—fourfold, while the results of (a) and (d) would indicate approximately twofold and threefold dilutions. These trials of potassium chloride as a standard were carried out on a uniform series of guinea-pigs, without any attempt to discover the extreme range of variation of the results obtainable by the method with one pituitary extract. The results are not selected, but recorded exactly as they were obtained. If we had desired to exhibit wider discrepancies we could doubtless have obtained them in a longer series. It seemed to us, however, that these four experiments, carefully conducted under the best conditions, showed that the expression obtained, in any one experiment, for the activity of pituitary extract in terms of potassium chloride, was purely accidental. We accordingly conclude, that uteri

from different guinea-pigs vary independently in their sensitiveness to potassium chloride and to pituitary extract respectively, and that these variations are of such an order that potassium chloride cannot be used as a standard for the activity of pituitary extract.

Incidentally we may note that the lowest estimate for the activity of our extract gives it rather more activity, in relation to the amount of dry gland substance represented, than that suggested by Spaeth for adoption as a standard strength. As mentioned above, our extract was made from old dried material, having only about one quarter of the activity present in the infundibular substance immediately after drying. Spaeth's suggested standard could not, therefore, in our opinion be, in any case, regarded as representing a proper level of activity for an extract purporting to be a 10 per cent. extract of fresh infundibular lobe, even if the indications obtained by his test were more constant for the same preparation.

Wide as the discrepancies are between our individual attempts to estimate the activity of the same extract in relation to either of these standards, we believe that they would have been much wider if we had varied the composition of the Ringer's solution. We regard as fundamentally unsound such a method as that proposed by Kochmann (1921), who reduces the calcium of his Ringer's solution and adds magnesium chloride, until even the uterus from an old guinea-pig no longer shows spontaneous contractions. He then proceeds to estimate the activity of a series of pituitary preparations by comparing the heights of the contractions produced by a standard dose of each, with a scale produced by varying doses of histamine. He gives no evidence of any precaution to ascertain that the effects of several successive doses of the *same* preparation would be identical. Under the conditions of his method we think this very improbable. This is an extreme example of faith in the mechanical accuracy of comparison obtainable between the pituitary extract and an artificial standard. In minor degrees, however, we believe that small variations in the composition of Ringer's solution, which would be very difficult to exclude from the practice of widely scattered laboratories, would introduce systematic differences between the estimates of different workers using the same artificial standard, in addition to the variations inseparable from the method. In our own series the composition of the Ringer's solution has been constant, the guinea-pigs have formed a reasonably uniform series as regards age and weight, all conditions have been kept rigidly the same, and all the experiments have been carried out in the space of a few weeks by the same practised workers. We do not think that conditions more favourable to the performance of the suggested standards could have been devised; yet we are driven to the conclusion that their use gives no closer estimate of the value of a pituitary extract than could be obtained by some simpler expedient, such as the determination of the highest dilution capable of producing a maximal contraction of the uterus. Such a method would obviously suffer from the large inaccuracy due to variation in the sensitiveness of different preparations; but if an extremely rough estimate were sufficient, we believe it would be better to choose a method suffering from such an obvious and frankly recognized inaccuracy, than to use standards which are

supposed to eliminate this source of error, but, in actual fact, give no trustworthy information concerning the sensitiveness of the preparation to the pituitary active principle. We do not regard it as impossible that, in due course, a stable chemical standard will be discovered which does give this information; if it were, we should strongly advocate its adoption. Meanwhile we hope to show, in the next section, that an empirical standard, prepared from the pituitary substance itself, can be made with reasonable uniformity; and that by its use it is already possible to make a much closer estimate of the activity of a pituitary extract, than with any of the artificial standards yet suggested.

V. THE USE OF AN EXTRACT MADE FROM THE PERFECTLY FRESH PITUITARY GLAND AS A STANDARD.

Our conclusion being that, at present, no satisfactory artificial standard can be found, we have to inquire whether any preparation of sufficiently certain uniformity can be made from the infundibular substance itself. The ideal standard, as Dale and Laidlaw pointed out, would be a dry, stable sample of the infundibular substance, or of some active preparation therefrom, from which an extract or solution could be prepared from time to time, according to an accurately prescribed routine. We are not without hope that such a preparation may eventually be available. Some years of observation will be required, however, before it is possible to claim absolute stability for such a standard. We know already that the infundibular substance, 'dried' in an ordinary commercial sense, and kept in the laboratory in a stoppered bottle, loses an important part of its activity in the space of a year or two. The question has still to be answered whether such material, if treated like a standard antitoxic serum, by drying to absolutely constant weight over phosphorus pentoxide, and sealing in perfectly dry vacuum, will remain stable. It is clear, however, that such a possibility cannot be put to the test unless some other kind of reasonably good standard is available for comparison. We believe that such can be found in the simplest possible kind of preparation, namely, the simple, heat-coagulated watery extract from the perfectly fresh infundibular substance of the ox pituitary body. Seasonal variations in the glands have often been alleged as a probable cause of the lack of uniformity in the strength of the commercial extracts. We can find no experimental basis for the suggestion, which seems to have arisen from an assumed analogy with other ductless glands. Fenger reports that he was unable to detect any such seasonal fluctuations in the activity of the pituitary posterior lobe. Our own experiments, definitely directed to this point, and extending over a period of many months, have convinced us that no variations, appreciable by the methods available, occur in the activity of the perfectly fresh material from the only species which we have investigated, namely the ox.

(1) *Method of Making the Extract.*

(a) *Procuring the material.* This was obtained direct from the slaughter-house, the glands, as soon as dissected, being handed to

a messenger who brought them straight to the laboratory. In our earlier series of comparisons we took no special precautions to keep the glands cold during this transit, so that they were, for about two hours, at the variable temperature of the air before being dissected and worked up. No differences were detected between the values of the extracts from glands thus treated, the comparison extending to six samples obtained at intervals between August and December. A seventh sample yielded an extract of distinctly lower activity, but the cause of this, which will be discussed later, was clearly ascertained, and had no connexion with changes in the glands between killing and extraction. It appeared to us unlikely, however, that no change occurred during the interval of exposure to a temperature of about 18°C . It seemed more probable that some deterioration had occurred but, under the conditions of collection, had been sufficiently constant to escape detection by the test. To ascertain whether this was so we had one small batch of glands divided into two lots immediately on receipt from the slaughterer. One lot was brought to the laboratory as before, without artificial cooling; the other lot was dropped at once into a wide test-tube, which was kept immersed in a freezing mixture contained in a Dewar (thermos) flask, so that the glands were quickly frozen and brought thus to the laboratory. Both lots were kept longer than usual in the laboratory, and when $3\frac{1}{2}$ hours had elapsed since removal from the carcase, they were dissected simultaneously, extracted in precisely the same manner, and tested against one another. The extract from the cooled glands had about 30 per cent. greater activity than that from those transported at air temperature. Since local variations, both of transmission-time and temperature, are bound to occur, we recommend that the standard extract should be made from glands which are cooled to near freezing point as soon as they are obtained from the carcase, and kept thus until they are dissected for extraction, the time between slaughter and extraction being, in any case, made as short as possible. We found that the posterior lobes from 6 to 8 glands gave a convenient volume of 'standard' extract. Smaller quantities are more difficult to handle; larger are undesirable for reasons to be indicated later.

(b) *Preparation for extraction.* The glands are dissected as rapidly as possible, care being taken that nothing but pure infundibular lobe substance is retained. Occasionally, in the region of the *pars intermedia*, small masses of colloid material are found. These should not be included in the material to be extracted, and they are easily detached. In one gland we found a piece of the colloid material sufficiently large to be worked up by itself into a separate small volume of extract, an extract being made under exactly parallel conditions from the posterior lobe of the same gland. The extract from the colloid material was entirely inert on uterus and blood pressure; that from the infundibular lobe was quite normal in activity. The observation is worthy of record, in criticism of the suggestion that the colloid, formed by the *pars intermedia*, represents the active principle, and that its presence in the posterior lobe, on its way to secretion, confers on the latter its physiological activity. It is quite clear that the colloid does not yield the active principle to the ordinary methods of extraction, and there is no reason to

suppose that it is in any way connected with the kinds of activity with which we are concerned. We found no difficulty in separating the posterior lobe, which shells out cleanly from such inert admixtures.

Strictly speaking, a standard method of extraction should include a standard of fineness for the subdivision of the material to be extracted. We have none to offer. Fenger tested the effect of careful mincing on the yield of active principle, and found, as might be expected, that fine division increased the yield of activity. If it should prove possible to make a stable, dry standard, this difficulty will not arise, since the dried material can be pulverized finely and perfect extraction secured. Meanwhile, in using the fresh, moist gland substance, we have not thought it desirable to add such aids to subdivision as sand, glass, or Kieselguhr, and therewith to introduce unknown possibilities of adsorption of the active principles. We have been content to take the 6 to 8 dissected posterior lobes and mince them together, with thorough mixing of their substance, by snipping as finely as possible with small dissecting scissors.

The material, thus minced and mixed as rapidly as possible, is weighed out into suitable portions for extraction. The weighing is conveniently done by taking the half of a microscopic glass slide, weighing this accurately, placing about 1.5 gm. of the minced gland substance on it, and weighing again.

(c) *Process of extraction.* The slide, with the weighed pituitary substance on it, is introduced into a 200 c.cm. flask, with a short wide neck. Distilled water is added in the proportion of 40 c.cm. to 1 gm. of gland substance. The latter is washed off the slide and distributed in the water by shaking, and the mixture, with occasional agitation, is allowed to stand for one hour at the laboratory temperature (i. e. not above 20° C.). At the end of this period of cold extraction a half normal solution of acetic acid is added, in the proportion of 0.2 c.cm. to 1 gm. of the gland substance. The neck of the flask is then plugged with cotton-wool and the belly is plunged into a *large* bath of *briskly boiling* water. The object is to bring the contents of the flask to a temperature near 100° C. in the minimum time. The flask is kept in the boiling water for ten minutes, and is then removed and cooled under the tap. The cooled extract is filtered through a folded paper, and the coagulum is not washed. The filtrate represents a '2.5 per cent. extract of fresh gland substance'.

Determinations carried out by our colleague Dr. H. W. Dudley showed, on two samples, ratios of 18.6 and 19.4 of dry substance to 100 of fresh substance. As a convention we have adopted 20 per cent. as expressing, with sufficient accuracy, the proportion of dry material present in a known weight of fresh gland. Our '2.5 per cent. extract of fresh gland' is, therefore, termed a '0.5 per cent. extract' in terms of dry gland substance. We have always diluted it tenfold with water, and distributed the dilution in a series of test-tubes. These, after plugging with cotton-wool, are placed in the boiling water bath for three minutes and then stored in the refrigerator until required. Such brief additional heating does not impair the activity perceptibly, and secures such relative sterility as is adequate for keeping the extract for a week or two in the refrigerator. The standard, as stored for brief periods in this

approximately sterile condition, represents, therefore, a 0.25 per cent. extract of the fresh, moist gland substance, or a 0.05 per cent. extract in terms of dry gland substance.

It is necessary to insist that, if uniformity of result is to be obtained, the method of extraction must be kept constant in every detail. Mention has already been made of the difference made by keeping the glands thoroughly chilled between the time of their removal and the preparation of the extract. We believe that a more thorough comminution of the material would probably result in more complete extraction, and might render the preliminary cold extraction unnecessary. It seems highly probable, also, that the deterioration detected in the undissected gland material at the ordinary temperature, likewise proceeds during the preliminary extraction of the fresh minced material with water at the room temperature. We have found, however, by experiment, that it is more than counterbalanced by the more perfect extraction which this preliminary soaking effects when the gland substance is simply minced as finely as possible with scissors. Admittedly this is a compromise; but it is a convenient one, and we have found it to give extracts of very regular activity when the indicated time limit is strictly observed.

A much more important source of variation, which does not so readily suggest itself, is the rate at which the suspension of gland substance in acidulated water is heated to the coagulation point. We came upon this source of error accidentally, though it might have been predicted by a more thorough consideration of the factors involved. The fall of activity, occurring slowly at the ordinary temperature, may be attributed with some confidence to the action of an autolytic enzyme. This again may be assumed to act with much greater rapidity at temperatures between 30° and 50° C. The longer the period of heating through this range, the greater the fall of activity which may be expected from this action. We have clear evidence of this deleterious effect of slow heating. In the directions given above, the size and shape of the vessel indicated, in relation to the volume of fluid, and the stipulation that the water bath—into which it is plunged—must be large and briskly boiling, are all designed to bring the mixture as rapidly as possible to a temperature above that at which autolytic enzymes are put out of action. In this way, as we have found by experience, we obtain the maximum activity which can be extracted from the glands under the given conditions with regard to relative volume of water, mincing of glands, &c. If we prescribed any but the minimum possible period for attaining the 'lethal' temperature, it would be necessary to prescribe not only a standard total time of heating up, but a uniform rate of rise, so that the time at each intervening temperature would be constant. This we regard as impracticable. We therefore choose, in this instance, the procedure giving the optimum preservation of activity, as being the easiest method of obtaining a *constant* result. As regards the other conditions—the volume of liquid in proportion to weight of gland substance, &c.—these are chosen for convenience, and fixed, not with the idea that they give us the whole of the activity of the gland substance, but that they give us a sufficiently *constant* proportion. Using a larger relative volume we should

probably secure greater completeness of extraction, subject, however, to a less constant loss by slower heating, unless the vessels used were inconveniently large.

No purpose would be served by an attempt to obtain the whole of the activity in solution, in making our standard. No process of manufacture on a large scale preserves or can be expected to preserve a proportion nearly so large as that obtained on a small scale by the above process. Our sole aim is to obtain a constant standard of reference, and we have reason to believe that this can be achieved by a rigid adhesion to the above directions.

(2) *Method of Performing the Test.*

The horn of a suitable uterus, having been carefully dissected and suspended in the bath of warm Ringer's solution, must be left to itself until relaxation is approximately complete. This will usually take from 20 to 30 minutes. Any necessary adjustment of the weight of the lever is then made. A first dose of the standard preparation is then given and the effect recorded. As a first dose we ordinarily use 0.5 c.cm. of a 50-fold dilution of the standard fresh extract. The undiluted extract represents, it will be remembered, 2.5 per cent. of the fresh infundibular substance, or, approximately, 0.5 per cent. of the dry weight; so that the 50-fold dilution is an 0.05 per cent. or 0.01 per cent. extract, according to the notation adopted. For convenience we have usually recorded the strength in terms of dry weight, assuming this to be one-fifth of the moist weight. We start, then, with a dose of 0.5 c.cm. of 0.01 per cent. extract, added to the bath containing 125 c.cm., giving a dilution of 1 part of original dry weight in $2\frac{1}{2}$ millions. The effect varies much with different samples of uterine muscle. With an initially sensitive preparation the result will be a contraction to a maximum, maintained for a few minutes. In such a case the contents of the bath are changed for fresh Ringer's solution and time allowed for complete relaxation. This process, after such a first dose, is frequently less rapid than at a later stage. After 15-20 minutes, however, the muscle should be relaxed and ready for a second dose, which, in such a case, is smaller than the first. After a few trials a dose is found—usually 0.3 to 0.4 c.cm. of the indicated dilution—to which the muscle responds regularly, with identical contractions which are less, but not widely less, than the maximum of which it is capable. In the case of a less sensitive preparation, especially with the delicate horn from the uterus of a very young guinea-pig, the first effect of 0.5 c.cm. of the dilution may be very small. In such a case it is well, after change to pure Ringer's solution and relaxation, to repeat the same dose. It will usually produce a much larger response. If after 3 or 4 such doses the response is still widely submaximal, the dose must be increased; but it is desirable to establish the fact, that the regular response of the accustomed muscle to such a dose is widely submaximal, before such an increase is made. The aim in any case is to establish, for the particular preparation, the dose which is about 60-70 per cent. of that needed to produce contraction to the maximum.

0.5% dry wt
= 0.01%
0.5 c.c. in 125
= 0.01
250
= 1:25000
= 1:25000

parison, makes a heavy demand on the patience of the observer. We are convinced, however, that results of real value can be obtained from this test in no other way. Occasionally the worker will, by good fortune, obtain a preparation which settles down to regular activity almost immediately; or a lucky shot at the appropriate dose of the unknown extract may enable him rapidly to establish an equivalent for the standard. In such ways the time needed for a test may be much reduced, but any attempt to accelerate the determination, by departure from the essential lines of the procedure above described, will, in the end, result in untrustworthy estimates and wasted time. It is, with us, a rare experience to have to abandon a test because the preparation will not give a response of sufficient constancy or a discrimination sufficiently fine. One determination, carefully and conscientiously carried through, without stint of time or patience, is of far more value in result, and far less expensive in material, or even in time, than six in which impatience has yielded a series of rough estimates.

VI. THE REGULAR ACTIVITY OF PERFECTLY FRESH GLANDS.

The experiments on this point were conducted between August 1921 and March 1922. As a standard of reference, having presumably an approximate stability, we used, on several occasions during the test, an extract made from the old specimen of dried infundibular substance, previously mentioned in connexion with the tests with histamine and potassium chloride. In this series, however, the dried material was only used for occasional confirmation of results obtained, mostly, by direct comparison of successive batches of extract from fresh glands with one another. In the case of the first two batches made, the interval—August to October—was considered too wide for such direct comparison to be trustworthy. The extract of the 18th of August had, in fact, been destroyed before that of the 11th of October was prepared. Comparison in this case was, therefore, made only with reference to the dry gland extract, which is called 'A' throughout these tests, the fresh gland extracts being called F_1 , F_2 , &c., in the order of the dates of preparation. Table I shows the details of the comparison of F_1 and F_2 with A.

TABLE I.

<i>Date of preparation.</i>	<i>Dilutions (in terms of dry material) used in the comparisons.</i>	<i>Equivalent doses of these dilutions.</i>	<i>Equivalents of activity.</i>
	<i>Fresh gland extract.</i>	<i>Extract 'A'—</i>	
F_1 Aug. 18/21	0.025 %	0.05 %	$0.3F_1 = 0.45A$
F_2 Oct. 11/21	0.01 %	0.02 %	$0.75F_2 = 1.125A$
			$F_1 = 3A$ $F_2 = 3A$

It will be seen that these two first preparations of the fresh gland extract were equal in activity, on the assumption that the old sample of dried gland lost no activity between these dates. In the case of the later extracts, which were prepared at shorter intervals, each was compared with its predecessor, F_4 being compared with 'A' as well. Table II shows the results (see also Fig. 8).

TABLE II.

<i>Extracts compared.</i>	<i>Dates of preparations.</i>	<i>Dilutions used in the test.</i>	<i>Doses found equivalent.</i>	<i>Equivalents of activity.</i>
F ₂ & F ₃	Oct. 11 & 18	0.01 %	0.8F ₂ = 0.8F ₃	F ₂ = F ₃
F ₃ & F ₄	Oct. 18 & 25	0.01 %	0.6F ₃ = 0.6F ₄	F ₃ = F ₄
F ₄ & A	Oct. 25	0.01 % F ₄ 0.05 % A	0.7F ₄ = 0.4A	F ₄ = 3A
F ₄ & F ₅	Oct. 25 & Nov. 29	0.01 %	0.5F ₄ = 0.5F ₅	F ₄ = F ₅
F ₅ & F ₆	Nov. 29 & Dec. 6	0.01 %	1.0F ₅ = 1.4F ₆	F ₅ = 1.4F ₆
F ₆ & F ₇	Dec. 6 & Dec. 13	0.01 %	0.5F ₆ = 0.35F ₇	F ₆ = 0.7F ₇ (or F ₇ = 1.4F ₆)

Since F₂ had previously been found equal in activity to F₁, it is seen that, out of the extracts made from seven samples of fresh glands, obtained between August and December, only F₆, the sample of the 6th of December, showed any departure from strict uniformity of activity. It formed such a remarkable exception that a careful scrutiny was made of the process of its preparation. It had happened that our colleague, Dr. Dudley, who made all the other extracts, was absent on this date, and that one of us (J. H. B.) had made this extract, with a supposedly strict observance of Dudley's routine. On investigation, the only discrepancy discovered was that Dudley had heated his extract, after the preliminary soaking in the cold, by plunging into a small boiling water bath, while Burn had placed it in the same water bath before this boiled, about 5 minutes being occupied in bringing it to the boil. It seemed probable that the rapid action of an autolytic ferment during the period of heating between 30° and 50° was responsible for this greatly inferior activity of the more slowly heated sample.

The next sample of fresh glands, F₈, was accordingly divided into two portions after mincing. Both were made up and treated in exactly the same way, up to the process of heating on the water bath. One extract was then heated as rapidly as possible, by plunging the relatively capacious round-bottomed flask containing it into a large bath of briskly boiling water. The other extract was placed in a cold water bath which was then heated, about 20 minutes being taken to reach the boiling point. The two extracts were then compared with one another and with F₇.

The test established the following equivalents :

$$\begin{aligned}
 F_{8a} \text{ (rapid heating)} &= 1.5 F_7. \\
 F_{8b} \text{ (slow heating)} &= 0.66 F_7. \\
 F_{8a} &= 2.25 F_{8b} \text{ (see Fig. 9).}
 \end{aligned}$$

It will be seen that F_{8a}, for which precautions were taken to secure very rapid heating to 100°, had 50 per cent. more activity than F₇, while the slowly heated F_{8b} fell even further below the activity level of F₇, than F₆, which was slowly heated by accident. We decided, therefore, to adopt the most rapid heating easily obtainable in the preparation of the standard extract, as the only means of obtaining a practical uniformity with respect to this important detail.

In later experiments the effect of chilling during transit from the slaughter-house, as already described, was twice tested, the result showing, in each case, a 30 per cent. superiority for the chilled specimen over that transported at ordinary temperature. The results

obtained in these experiments were not strictly comparable with those of the earlier extracts from glands transported at the ordinary temperature, since the interval between slaughter and extraction was deliberately lengthened, in order to make obvious any superiority due to transport below 0°C . These last experiments, moreover, were carried out at an interval of 3 months after the latest of the earlier series, and it was not thought desirable to make a direct comparison of the new extract with one which had been kept so long, even at 0°C . There was, however, no obvious departure from the level maintained by the earlier fresh extracts, as judged by comparison with extracts from the old sample of dried material which has been several times mentioned.

Our conclusion is, therefore, that extracts of perfectly fresh infundibular material, if prepared by a method kept exactly constant in all its details, furnish a sufficiently constant standard of reference for the biological testing of pituitary extracts.

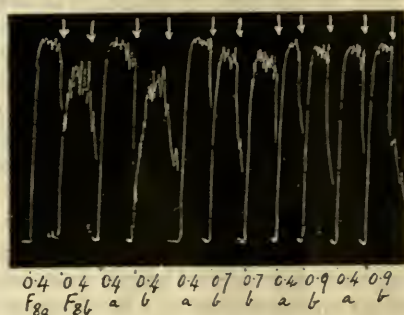


FIG. 9. The tracing shows the comparison between the fresh gland extract F_{8a} and F_{8b} (see text). For testing, a 50-fold dilution of a 2.5 per cent. extract of fresh gland was used, and the figures below the tracing represent the amount of each extract in c.cm., added to the bath of 125 c.cm. It is seen that 0.4 c.cm. of the extract prepared by 'rapid heating' is as active as 0.9 c.cm. of the extract of the same glands prepared by 'slow heating'.

VII. THE ACTIVITY OF COMMERCIAL EXTRACTS.

From the demonstration, given in the previous section, of the strictly constant activity of extracts from the perfectly fresh gland material, made by a uniform conservative method, it is an obvious corollary that, if all extracts made for therapeutic use could be prepared under such conditions, no standardization would be necessary, or, indeed, possible. Each batch of extract would be, in itself, the standard which we propose to apply.

The fact has to be faced, however, that it would be impossible, on these lines, to meet the world's demand for this preparation. Small-scale working is of the essence of the method which we have recommended for the preparation of the 'Standard fresh gland extract'. For good or ill, the use of the pituitary extract, particularly in obstetrics, has grown with such rapidity and to such dimensions, that the supply of raw material is scarcely adequate to meet the demand. As in the case of other pharmaceutical preparations, we have to accept organized mass-production, with its disadvantages as well as its convenience and efficiency.

Several of the precautions, which we found essential for the production of extracts of uniformly high activity, are hardly applicable to large-scale working. Even where the slaughtering and the working up of the carcasses are centralized and organized, the time between the death of the animal and the completion of the dissection process will probably be materially longer than in a laboratory, where only six glands have to be dissected.

In other cases there is no room for doubt that the time between slaughtering and working up may vary very widely, some makers having to depend for their supplies of glands upon scattered and distant sources. In many cases economy in working will be favoured by making extracts in bulk, at intervals not too frequent. This necessitates the accumulation and storage of gland material in the dried form, to be worked up in accordance with demand. It is possible that the activity of gland substance, if dried absolutely to constant weight, may be indefinitely stable. It is certain that that of the substance, dried in the ordinary, factory sense, is not so. Further, the heating up of a large volume of the watery suspension to coagulation point cannot, if carried out by ordinary methods, have the momentary brevity which we found essential to the preservation of the activity. Under ordinary conditions, the time taken to reach the temperature of safety for the active principles may be expected to vary with the volume to be heated.

For all these reasons, it was not to be expected that any of the extracts on the market would exhibit such a high proportion of activity, in relation to the weight of gland substance used, as those which we prepared on the small scale. We have not, in fact, found any which does so. It is obvious, also, that variations in the activity of the finished product are to be expected, unless the 'physiological standardization' applied by the makers has eliminated them.

Before we proceed to the evidence concerning the activity of commercial extracts, we may dispose of one additional point. We mentioned above the imperfect stability of incompletely dried material, as a possible source of defective or variable activity in commercial extracts. The question arose, however, whether the drying process, in itself, could be effected without loss.

(1) *Effect of Drying on Activity.*

The drying and the preparation of the equivalent extracts were kindly carried out for us by our colleague, Dr. H. W. Dudley.

We found that drying the fresh gland material over phosphorus pentoxide *in vacuo* was not accompanied by any loss of activity, whether the drying was carried out at 0° C. or 40° C. In the latter case the drying took place very rapidly, and this presumably compensated for any greater tendency to deterioration of the moist material at the higher temperature, which might have been expected. On the other hand, drying at room temperature *in vacuo* over sulphuric acid was accompanied by a significant loss of activity. We have no clear evidence as to the cause of the loss under these conditions. Our object, however, was to discover whether drying, in itself, was necessarily accompanied by loss, and this question is

answered in the negative by the experiments on drying over phosphorus pentoxide.

The following are the details of the comparisons :

1. Equivalent extracts made directly from fresh glands (F) and from the same material dried at 0°C . over P_2O_5 (D_1). Tested in dilutions representing 0.05 per cent. of dried material.

$$0.4 \text{ c.cm. F} = 0.4 \text{ c.cm. D}_1.$$

2. Equivalent extracts made from fresh glands (F) and from material dried over H_2SO_4 at 15°C . (D_2). Tested in dilutions representing 0.01 per cent. of dried material.

$$0.75 \text{ c.cm. F} = 1.12 \text{ c.cm. D}_2.$$

3. Equivalent extracts made from fresh glands (F) and from material dried over P_2O_5 at 40°C . (D_3).

$$1.0 \text{ c.cm. F} = 0.8 \text{ c.cm. D}_3.$$

It will be seen that, in the last experiment, the extract from dried material was slightly but distinctly more active than that from the corresponding fresh material. Presumably the drying facilitated a slightly more complete extraction of the active principle.

(2) *Examination of Commercial Samples.*

Samples of five different makes of the extract, which are current on the English market, were obtained directly from the manufacturers. Three were of British and two of American manufacture. In response to our request, the manufacturers, in all cases, courteously gave us confidential information as to the methods of manufacture and standardization. The following are the details, relevant to our estimates of the activity of these preparations, which were furnished with the samples supplied to us by the manufacturers as representative of their respective products.

Preparation P. Made from dry material by a special process of extraction. The strength arrived at is such as to represent a 20 per cent. extract of the fresh infundibular substance. The extract is made, in the first place, stronger than this. It is then physiologically tested in comparison with another well-known commercial preparation, the test on the blood pressure, and, more recently, that on the uterus, being employed. The activity of the extract is then adjusted by dilution. The sample supplied to us corresponded, after this adjustment, to a 5.6 per cent. extract of dry substance.

Preparation Q. This is stated to represent approximately a 20 per cent. extract of the fresh gland substance. The extract, however, is made from the dried material, and, according to the figures given to us by the manufacturers, in the preparation of the sample supplied, one part of this was taken to represent *six* parts of the fresh substance, instead of *five* parts, as we have assumed in most of our calculations. The preparation is standardized by the uterus method.

Preparation R. Made from dried glands, or from fresh glands frozen and kept in cold storage. Theoretical strength, a 10 per cent. extract of fresh infundibular substance. Standardized by uterus method, and by the blood-pressure method.

Preparation S. This is stated to be a 10 per cent. extract prepared from fresh infundibular substance. It is standardized by the uterus method.

Preparation T. This also is stated to be a 10 per cent. extract of the fresh infundibular substance. Standardization is carried out by the test on the blood pressure, though it appears that a test on the uterus is sometimes used as a control.

It should be noted that, whilst two of the preparations in the above list are of American manufacture, in no instance is reliance placed on the test with histamine as a standard, prescribed by the United States Pharmacopoeia. In two instances mention has been made to us of the use of this test as a supplementary control, but for their own satisfaction the makers, even in using the test on the uterus, appear in all cases to have used some pituitary preparation as a standard. In one case a previous satisfactory batch is used, in one a 'standard' extract, frequently renewed, and in one a 'specially prepared, highly active, stable powder'. The experience of manufacturers, therefore, has already brought their practice into accord with the suggestion made by Dale and Laidlaw, and strongly supported by our own recent experience, here recorded, that the only suitable standard yet available for the activity of pituitary extract is a preparation of the pituitary gland itself. That this is the case when methods based on the pressor action are concerned, had never been called in question.

There are two different questions which can be decided by comparison of the activity of these different commercial extracts with the standard fresh extract, and with one another. In the first place, we can find, in each case, what proportion of the activity originally present in the gland substance used in the preparation is still present in the extract as marketed. In the second place we can determine the relative activities of the different preparations to one another, irrespective of the amount of gland substance used. The two questions are not identical, because there is, among the manufacturers whose products we have examined, no agreement as to the proportion of gland to be used. In the United States the Pharmacopoeia has attempted to fix the proportion, in such a way that the preparation should represent a 10 per cent. extract of fresh infundibular substance. In this country some workers aim at a 20 per cent. extract; but two samples having this ostensible value were made from dry infundibulum in the respective proportions of 5.6 and 3.5 per cent. We may take the results bearing on these two points separately.

It will be seen that we have not, for all the commercial samples examined, figures of direct comparison with what we have termed the 'Standard Fresh Extract'. The experiments, by which we arrived at the suggested conditions for the preparation of this extract, were proceeding at the same time as the examination of the earlier samples supplied by the manufacturers. In some cases, therefore, we only made a determination of the value of the sample in terms of one of the earlier fresh extracts, in other cases in terms of an extract made, under Standard conditions, from the old dried material, which we have called extract 'A' above. Since, however, we have accurate records of the value of these in terms of

the Standard now proposed, a very simple calculation gives us the activity, of the manufacturers' samples in question, in relation to this Standard. In some other cases we have only determined the value of a sample in relation to one or more of the other commercial samples. Since, however, the relation of these to the Standard was already known, the value of the unknown sample in terms of the Standard could easily be calculated with an accuracy sufficient for our purpose.

(3) *The Proportion of the Original Activity present in the Finished Products.*

A word is needed as to the basis of calculation used in these comparisons. It is desirable, for simplicity, to express the theoretical strengths of all the extracts compared in terms either of the weight of fresh moist infundibular substance, or of the weight of dry substance represented. If all the extracts had been simply labelled in terms of fresh gland substance, this might have furnished the more convenient notation, our own Standard extract being made from the fresh material. We had, however, at our disposal definite information as to the proportionate weights of the dry material used in preparing some of the extracts, and the figures made it clear that estimates of the activity preserved, based on an assumed identity of the proportion of gland substance, would in these cases be misleading. It seemed unavoidable, in these cases, to express the yield in terms of the known weight of dry substance. This, however, involved the expression of the strengths of the other extracts under test, and of our Standard extract, in terms of dry substance, though some of them were made from the fresh material, and in others the weight of dry material was not accurately known. We have adopted what is, frankly, a conventional notation, in reckoning, in all such cases, that 5 parts of fresh gland substance are equivalent to 1 part of dry. Thus our Standard fresh extract is actually a 2.5 per cent. extract of the *fresh* gland substance, but we call it, for purposes of comparison, 0.5 per cent. extract of *dry* substance; similarly a commercial sample described as a 10 per cent. extract of *fresh* gland substance is reckoned as a 2 per cent. extract of *dry* substance. We are aware that this assumption is not strictly accurate, but it seemed to afford the best method of arriving at a common basis, and it will be seen that the differences to which we attach significance, in comparing the yields of activity, are of such an order that this minor inaccuracy of calculation could not affect them.

Preparation P. Sample 1. This preparation, being theoretically a 20 per cent. extract of fresh substance, and actually a 5.6 per cent. extract of dried substance, was diluted 200 times, giving a 0.028 per cent. extract of dried substance. It was compared against F_4 , which was diluted $2\frac{1}{2}$ times. F_4 being made to represent a 0.05 per cent. extract of dry material, on the assumption that 1 part of dry corresponds to 5 parts of fresh gland, our dilution is an extract representing 0.02 per cent. of dry gland, as obtained by the method used for F_4 . The equivalent doses, when added to the bath containing 125 c.cm., were

$$0.25 \text{ c.cm. P (0.028 per cent.)} = 0.3 \text{ c.cm. } F_4 \text{ (0.02 per cent.)}.$$

Therefore, at theoretically equal concentrations,

$$7 P = 6 F_4$$

$$\text{or } P = \frac{6}{7} F_4.$$

Now F_4 has only 70 per cent. of the activity of the corresponding fresh extract, prepared under the standard conditions now proposed. Therefore, the value of P , expressed in percentage of that of a 'standard extract' from the same amount of gland, is

$$\frac{6}{7} \times \frac{70}{1} = 60 \text{ per cent.}$$

That is to say, in making this sample of P from dried material, the manufacturers obtained 60 per cent. of the activity which can be extracted from the equivalent of fresh (or carefully dried) material by our standard method.

This same sample of P was also compared against Extract A , made from the old dried material, and the following equivalent was obtained :

$$0.4 \text{ c.cm. } P (0.014 \text{ per cent.}) = 0.55 \text{ c.cm. } A (0.025 \text{ per cent.}).$$

$$\therefore 56 P = 137.5 A.$$

$$P = 2.45 A.$$

$$\text{But } A = \frac{1}{4} \text{ Standard. (See under Q.)}$$

$$\therefore P = \frac{2.45}{4} \times 100 = 61 \text{ per cent. Standard.}$$

The two comparisons give practically identical values for this sample.

Sample 2. We had no exact data as to the weight of dry gland used, so that we have taken the 20 per cent. extract of fresh gland to represent a 4 per cent. extract of dry gland. The comparison was made with Standard fresh extract, with the following result :

$$0.7 \text{ c.cm. } P (0.02 \text{ per cent.}) = 0.7 \text{ c.cm. Standard } (0.01 \text{ per cent.}).$$

$$\text{i. e. } 2 P = 1 \text{ Standard}$$

$$\text{or } P = 50 \text{ per cent. of Standard.}$$

Preparation Q. Sample 1. This also theoretically represents a 20 per cent. extract of fresh substance, but is actually a 3.5 per cent. extract of dried substance. It was first tested against Extract A , made from the old dried powder. The extract made from this, under standard conditions, has been repeatedly found to have almost exactly one quarter of the activity of the standard fresh extract. The dilutions of Q used for testing were a 200 times and a 400 times dilution, with dry gland equivalents of 0.0175 and 0.00875 per cent. respectively. Extract A was used in dilutions corresponding to 0.025 and 0.05 per cent. of dry substance.

The following were the equivalents found :

$$(1) 1.2 \text{ c.cm. } Q (0.00875 \text{ per cent.}) = 1.0 \text{ c.cm. } A (0.025 \text{ per cent.}).$$

$$(2) 0.6 \text{ c.cm. } Q (0.0175 \text{ per cent.}) = 0.4 \text{ c.cm. } A (0.05 \text{ per cent.}).$$

(3) In Experiment (2) a further comparison was made between A and F_4 , with the following result :

$$.4 A (0.05 \text{ per cent.}) = 0.7 F_4 (0.01 \text{ per cent.}).$$

$$\text{i. e. } 20 A = 7 F_4$$

$$\text{or } A = \frac{7}{20} F_4.$$

$$\text{But } F_4 = \frac{7}{10} \text{ of Standard}$$

$$\therefore A = \frac{7}{20} \times \frac{7}{10} = \text{practically } \frac{1}{2} \text{ Standard.}$$

$$\text{From (1) } 105 Q = 250 A.$$

$$\text{or } Q = 2.4 A.$$

$$\text{From (2) } 105 Q = 200 A$$

$$\text{or } Q = 1.9 A.$$

The correspondence is not very good. If we take as average

$$Q = 2.2 A,$$

$$\text{then } Q = \frac{2.2}{4} \times \frac{100}{1} = 55 \text{ per cent. of the Standard.}$$

Sample 2. The second sample, examined some six months later, was compared directly with standard fresh extract. We had no accurate indication of the proportion of dried gland used for this sample. We have assumed, as in other cases, a ratio of 1 : 5, the dilutions of the sample Q and of the standard being expressed in dry gland substance on this basis. The following was the equivalent obtained :

$$1.0 \text{ c.cm. } Q (0.02 \text{ per cent.}) = 0.25 \text{ c.cm. Standard } (0.05 \text{ per cent.}).$$

$$\text{i. e. } 200 Q = 125 \text{ Standard.}$$

$$\therefore Q = 62.5 \text{ per cent. of Standard.}$$

Preparation R. This theoretically represents a 10 per cent. extract of fresh gland substance, though it is sometimes made from dried gland, in a proportion not exactly known to us. We have again assumed a ratio of 1 : 5 for dry weight to fresh weight.

Sample 1. This sample was first compared twice with Extract A, and the equivalents obtained gave us

$$(1) R = 1.9 A.$$

$$(2) R = 2.1 A.$$

$$\text{Average } R = 2.0 A.$$

$$\text{Since } A = \frac{1}{4} \text{ Standard, } R = \frac{2}{4} \times 100 = 50 \text{ per cent. of Standard.}$$

Six months later the same sample was compared with the Standard fresh extract, with the following result :

$$0.45 \text{ c.cm. } R (0.02 \text{ per cent.}) = 0.5 \text{ c.cm. Standard } (0.01 \text{ per cent.})$$

(See Fig. 10.)

$$\text{i. e. } 9 R = 5 \text{ Standard.}$$

$$R = 55 \text{ per cent. of Standard.}$$

These tests, which show a fair correspondence, indicate that in making this first sample of R the manufacturers obtained practically the same proportionate yield of activity as the makers of Q in preparing the first sample of that product.

Sample 2. This was compared only with the Standard fresh extract, with the following result :

0.5 c.cm. R (0.02 per cent.) = 0.6 c.cm. Standard (0.01 per cent.).

i. e. 10 R = 6 Standard

$\therefore$ R = 60 per cent. Standard.

Preparation S. This is said to be a 10 per cent. extract prepared from fresh infundibular substance.¹ The ratio 1 : 5 for dry to fresh weight was assumed. A few preliminary trials showed a very definite inferiority to the extracts previously examined, in theoretically equivalent dilutions. The value of S has not been measured directly in relation to the Standard, but its relation to the first samples of P and R was carefully determined, these being diluted according to the indications given above.

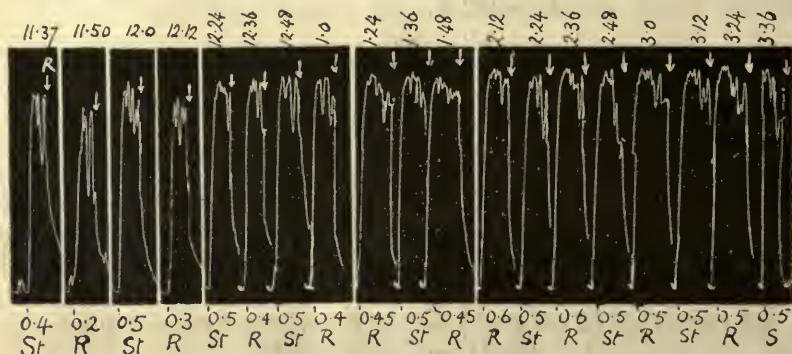


FIG. 10. A comparison of a commercial preparation (R) with our Standard fresh gland extract (St).

The Standard is a 50-fold dilution of a 2.5 per cent. extract of the fresh glands, and this dilution represents a 0.01 per cent. extract of the dry material present. 'R' is theoretically a 10 per cent. extract of fresh gland substance; it was diluted 100 times; this dilution represents a 0.02 per cent. of the dry material present, so that the dilution is theoretically twice as strong as that made from the Standard extract. The tracing shows that 0.45 c.cm. R = 0.5 c.cm. St, so that R contains activity = 55 per cent. of St. The figures above the tracing are the times at which the corresponding doses were put into the bath.

(1) 0.5 c.cm. S (0.04 per cent.) = 0.5 c.cm. P (0.014 per cent.).

(S being diluted 50 times, P 400 times.)

i. e. 20 S = 7 P

or S = 0.35 P.

Since P = 60 per cent. S = 21 per cent. Standard.

(2) 0.7 c.cm. S (0.02 per cent.) = 0.3 c.cm. R (0.02 per cent.). (See Fig. 11.)

i. e. $S = \frac{3R}{7}$

Since R = 50 per cent. $S = \frac{3 \times 50}{7} = 21$ per cent. Standard.

¹ Subsequent information suggests that we may, by mistake, have been supplied with a sample representing a 6 per cent. extract of the fresh gland, instead of 10 per cent. as originally stated. If this were the case the figure for the percentage yield of available activity should be 35 instead of 21 per cent. for preparation S. In the legend to Fig. 11, R should, in that case, be 1.4 times, instead of 'more than twice' as strong as S; in Table III the figure for S would be 35.

Both these comparisons, therefore, give the same value for S in relation to the Standard.

Preparation T. This is also said to be a 10 per cent. extract of the fresh infundibular substance. It was tested on several occasions against P, Q, and R, the results all showing a very low activity for T. The comparison against R may be quoted. Assuming a 1:5 ratio

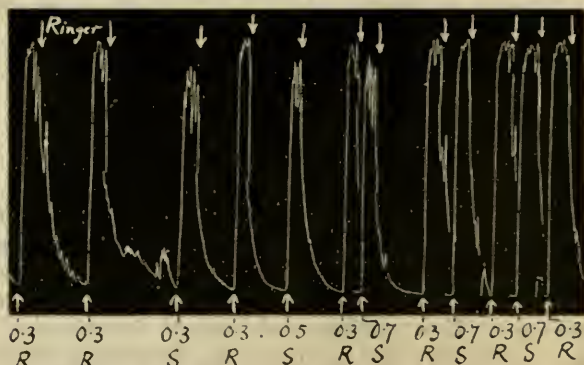


FIG. 11. Comparison of two commercial preparations R and S. Both represented 10 per cent. extracts of fresh gland substance, and both were diluted 100 times for testing. So far from equal doses producing contractions of the same height, the tracing shows that a dose of 0.3 c.cm. R is equivalent to 0.7 c.cm. S, so that R is more than twice¹ as strong as S.

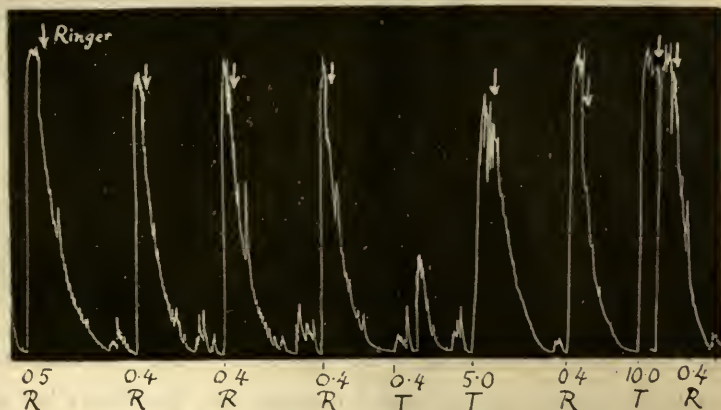


FIG. 12. Comparison of commercial extracts R and T. Both represented 10 per cent. extracts of fresh glands. When both were diluted 100 times, 0.4 c.cm. R gave a good contraction, but 0.4 c.cm. T produced no effect whatever. A fresh sample of T was taken and diluted only 10 times, and 0.5 c.cm. of this gave the response recorded in the tracing as 5.0 T. Finally 1.0 c.cm. of a 10-fold dilution of T was approximately equal to 0.4 c.cm. of a 100-fold dilution of R.

for dry to fresh weight in each case, and diluting T 10 fold and R 100 fold, we found:

1 c.cm. T (0.02 per cent.) = 0.4 c.cm. R (0.02 per cent.). (See Fig. 12.)
So that T = 0.04 R.

Since R = 50 per cent. Standard, T = 2 per cent. Standard.

These various results may be tabulated. If we assume that our

¹ Or 1.4 times. See footnote, p. 44.

standard method extracts the whole of the activity of the perfectly fresh gland substance, the following are the percentages, present in the final products, of the activity originally present in the gland substance used in making these different samples :

TABLE III.

<i>Preparation.</i>	<i>Proportion of activity present in final product. (Standard = 100).</i>
P. 1st sample	60
2nd „	50
Q. 1st sample	55
2nd „	62.5
R. 1st sample	50 (or 55)
2nd „	60
S.	21 ¹
T.	2

It will be seen that P, Q, and R form a group in which the amount of original activity preserved varies between about 50 and 60 per cent. In the manufacture of S a much more serious loss—nearly 80 per cent²—has occurred, while in the case of T only a trace of the activity remains. It is, perhaps, of interest to note that S and T were samples of the only two preparations, concerning which we have been informed that they are wholly prepared from the fresh gland substance. The manufacturers of R state that they use fresh as well as dry material, but we have no information as to the form in which the gland was extracted in preparing the samples of R which we have tested. The poor activity of the preparations which we know to have been made from fresh glands may be a coincidence. On the other hand, it is possible that the activity of autolytic enzymes, during the heating up of a large volume, may play a more serious part in lowering activity when fresh material is used. We presume that, when finely powdered dry material is used, this could be added directly to water already at a temperature above the danger zone ; such a procedure would probably be inapplicable to the sticky mass of pulped fresh material. These, however, are purely speculative suggestions, and our only concern is to draw the attention of the makers to this possibility of a serious loss of activity in preparation, even when perfectly fresh material is used.

(4) *The Relative Activities of the different Commercial Extracts.*

While the results given above indicate the possibility of wide discrepancies between the amounts of activity obtained by different makers from the same quantities of gland material, they do not reveal the full extent of the variation in activity among the different extracts presented for therapeutic use. The labels, indeed, in some cases give indications of the theoretical strengths of the extracts. This, however, is not always done. In any case we have reason to believe that the medical practitioner, finding two different preparations of the pituitary gland, both dispensed in sealed ampoules containing 1 c.cm., usually assumes that they are equivalent, or intended to be so. We have, further, mentioned above the fact that the proportions of dry gland substance used in preparing even

¹ Or 35. See footnote, p. 44.

² Or 65 per cent. See footnote, p. 44.

theoretically equivalent extracts are by no means necessarily identical with different makers. If we take the various extracts, as the majority of practitioners do, as being theoretically equivalent, and compare them merely with regard to their activities, without any reference to the amount of gland represented, the contrasts are even more glaring than those tabulated above. In addition to the comparison of S and T with other preparations, already quoted, we may record the following direct comparisons of R with P and Q.

In all cases the comparisons refer to the first samples of these preparations tested :

$$(1) \ 0.25 \text{ c.cm. P (dil. 200 fold) } = 0.4 \text{ R (dil. 100 fold).}$$

$$\text{i. e. at equal dilutions, P} = 3.2 \text{ R.}$$

A very similar relation is obtained from the figures given earlier. P is a 5.6 per cent. extract, containing 60 per cent. of the available activity. R, on the assumption that it is a 2 per cent. extract (of dried material), shows 50 per cent. of available activity. This gives the activities

$$\text{P : R} :: 5.6 \times 60 : 2 \times 50$$

$$\text{i. e. P} = 3.36 \text{ R.}$$

$$(2) \ 0.5 \text{ c.cm. R (dil. 100 times) } = 0.55 \text{ c.cm. Q (dil. 200 times).}$$

$$\text{i. e. at equal dilutions Q} = 1.8 \text{ R.}$$

Again, from earlier comparisons, R, when taken as a 2 per cent. extract, shows 50 per cent. of available activity, and Q, a 3.5 per cent. extract, shows 55 per cent.

$$\text{Whence Q : R} :: 3.5 \times 55 : 2 \times 50$$

$$\text{i. e. Q} = 1.9 \text{ R.}$$

We have already, from direct comparisons, for S and T, which like R theoretically represent 10 per cent. extracts of fresh substance,

$$\text{S} = 0.43 \text{ R}$$

$$\text{T} = 0.04 \text{ R.}$$

We have, therefore, expressions for the relative activities of all these first samples, supplied directly by the manufacturers as representative, in terms of one of them, that of preparation R. If we represent the activity of the weakest, T, as unity, we obtain the following relative values.

TABLE IV.

The figures show the relative activities of the representative samples of infundibular extract from five different makers.

$$\begin{array}{l} \text{T} = 1 \\ \text{S} = 11 \\ \text{R} = 25 \\ \text{Q} = 45 \\ \text{P} = 80 \end{array}$$

(5) *The Comparative Pressor Activities of the above Samples.*

As already mentioned, we have not succeeded in obtaining results of the same quantitative accuracy with the test on the blood pressure as with that on the isolated uterus. Hamilton carries out the blood-pressure test on the dog under chloretone. We have used

the cat, pithed under preliminary anaesthesia with ether, and then kept under artificial respiration with pure air. We regard the results as sufficiently accurate to detect only the occurrence of any wide disproportion in the extent to which the pressor and oxytocic principles have been preserved.

1. Comparison of the fresh extract F_2 with extract A, made from an old sample of dried infundibulum. The test on the isolated uterus has shown that $F_2 = 3 A$. On the blood pressure equal rises were obtained with 0.5 c.cm. F_2 (0.1 per cent. dry gland) and 0.3 c.cm. A (0.5 per cent.)

$$\begin{aligned} 5 F_2 &= 15 A. \\ \text{or } F_2 &= 3 A. \end{aligned}$$

2. Comparison of samples P and Q. (By uterus test.)

$$P : Q :: 80 : 45.$$

For the blood-pressure test both extracts were diluted 16 times.

$$0.25 \text{ c.cm. } Q < 0.25 \text{ c.cm. } P.$$

No exact match was obtained, but P was apparently about twice as strong as Q—a result which is not discordant with that on the uterus.

3. Comparison of samples P and R.

At equal dilutions (8 times)

$$\begin{aligned} 0.25 \text{ c.cm. } P &> 1.0 \text{ c.cm. } R. \\ \text{or } P &> 4 R. \end{aligned}$$

This is not in full accord with the result of the tests of the uterus, which gave $P = 3.2 R$.

A second test on the blood pressure gave, with equal dilutions (16 times)

$$\begin{aligned} 0.75 \text{ c.cm. } P &= 2.0 \text{ c.cm. } R. \\ P &= 2.66 R. \end{aligned}$$

So that the two tests on the blood pressure give values for the ratio $P : R$ which lie on either side of that given by the tests on the uterus.

4. Comparison of samples P and S.

At equal dilutions

$$0.25 \text{ c.cm. } P > 0.7 \text{ c.cm. } S$$

and, later,

$$0.6 \text{ c.cm. } P < 1.6 \text{ c.cm. } S.$$

i. e., 0.25 c.cm. P first produces a somewhat larger effect than 0.7 c.cm. S and later a somewhat smaller one than 0.66 c.cm. S.

If we take 0.7 c.cm. S as the approximate equivalent of 0.25 c.cm. P,

$$P = 3.5 S.$$

This is a clearly different ratio from that obtained from the test on the uterus, which gave

$$P : S :: 80 : 11.$$

5. Preparation T. This, which had a very low degree of activity on the uterus, had no pressor action at all. Its effect on the blood pressure, in any dose, was purely depressant.

Since preparation T has no pressor action, we cannot, as in the case of the activities on the uterus, take it as unity. We can, however, make a rough comparison of the relative values obtained by the two methods, if we equate the pressor action of the strongest preparation P with its oxytocic activity, calling both 80. (Table V).

TABLE V.

<i>Preparation. (Manufacturers' samples).</i>	<i>Relative oxytocic activities, taking</i>	<i>Relative pressor activities, taking</i>
	<i>T = 1.</i>	<i>P = 80.</i>
P	80	80
Q	45	<i>circa</i> 40
R	25	15-30
S	11	<i>circa</i> 23
T	1	0

VIII. CONCLUDING REMARKS.

The figures given above speak for themselves. The infundibular extract is a remedy which is at once valuable and dangerous, and the fact that such variations can occur in the activity of preparations, habitually regarded by the medical profession as equivalent, is deplorable. We have purposely abstained from giving the names of the manufacturers concerned, or details which would enable their products to be identified from our descriptions. We have adopted this policy for two reasons. In the first place we do not take the view that the manufacturers are chiefly to blame for the position which we have displayed. No official guidance has been given to them in this country as to the strength of extract most suitable for therapeutic use, and no standard of activity has been available, though all the extracts we have examined were described as 'physiologically standardized'. We are satisfied that in all cases a conscientious attempt has been made to control the activity by a physiological test; we gather that in the cases of two members of the above series, the testing was not carried out by the manufacturers' own staff, but by external and presumably independent authorities. We thought it our duty to draw the maker's attention to the quality of the preparation which we have called T, and have since received from the same source preparations corresponding sufficiently well in activity with others, such as R, having the same theoretical strength.

In the second place, past experience seems to indicate that the publication of the names of makers, in connexion with figures showing the activities of their preparations, may result in advertising claims which are not truly justifiable. We are by no means convinced that the sample of P, in the above list, is a better preparation than that of R. The proportion of available activity extracted in its manufacture is but little greater; its much greater activity is due to the fact that nearly three times as much gland is represented in the same volume. To the practitioner accustomed to R, P would be dangerously strong, while one accustomed to P would find R unduly weak; but the manufacturer would not, in either case, be entitled to claim superiority for his product.

The position revealed by Table V is not only deplorable; we are convinced that it is quite unnecessary. It will be clear, from

what has been already said, that two different factors are involved in the variation. (1) The proportion of gland substance, of which the activity is supposed to be represented, is different in the case of different preparations. (2) The actual yield of activity, obtained from the same weight of gland substance, is widely different in the case of different preparations. We may take the two points separately.

(1) To secure uniformity of *theoretical* strength, nothing more is needed than the adoption of an agreed formula. For reasons already given, we do not think that the need is properly met by an indication, such as most manufacturers formerly gave, of the amount of gland substance represented in a given volume of each preparation. The medical man, in the hurry of an emergency, will not stop to read labels and make calculations. Whatever the preparation available, he will give the dose of 'pituitary extract' which he has previously found effective. The fact that several manufacturers have now abandoned the custom of stating the supposed strength of their extracts, suggests that they have learned that it has no value as a guide to dosage. The omission, however, makes the position perfectly clear. We may have two manufacturers issuing, with the same indications and for the same purposes, preparations of which one is theoretically twice as strong as the other; one of them will have the advantage of being able to make twice as much extract as the other from the same quantity of gland, while the other will, perhaps, find grounds for a claim that his preparation has 'unique' or 'exceptional' potency—a claim which ought properly to be regarded as a severe condemnation of his product. What is wanted is not competition to produce the greatest possible activity in proprietary preparations, but conformity of all makers to a common standard. By whomever it is prepared, the pituitary extract is, essentially, a sterilized watery decoction of the infundibular substance, and we can see no proper obstacle to agreement on a uniform theoretical strength for this decoction.

The United States Pharmacopoeia has officially settled this theoretical strength at 10 per cent. of the fresh infundibular substance. The British Pharmacopoeia has not yet recognized the existence of this potent remedy. We strongly recommend the general adoption, by British manufacturers, of the *theoretical* strength laid down by the United States Pharmacopoeia, so that the practitioner may be justified in his usual assumption, that the dose contained in the same volume is, at least, supposed to be the same, whatever preparation he uses.

(2) If this recommendation is adopted, there is further needed a physiological assay, to ensure that the proportion of activity extracted by different makers from the standard proportion of gland shall be reasonably uniform. The United States Pharmacopoeia has attempted to meet this need also. Unfortunately, as we have shown above, it has prescribed a standard which, so far as it can be applied at all, would result in the production of an extract having only a small fraction of the activity present in a 10 per cent. decoction of fresh gland substance, or of the equivalent of substance carefully dried when fresh. If the extract is defined as a 10 per cent. extract,

the standard of activity demanded should be that obtained in such an extract by the manufacturer who uses sound material and proper skill. In proposing as the standard of reference our 'Standard fresh extract', prepared on a small scale, we do not suggest that preparations nearly approaching it in activity, in corresponding dilutions, could be prepared on the manufacturing scale with any regularity. We have clear evidence to the contrary; and if they cannot be produced with regularity, they should not, if occasionally produced, be placed on the market without dilution. Our measurements seem to indicate 60 per cent. of the activity of our Standard extract, as a level which should be without difficulty attainable by any manufacturer who has the necessary equipment and staff for the careful extraction of perfectly fresh material, or of material which has been dried at low temperature when perfectly fresh, and used without unduly long storage. A 10 per cent. extract would need four-fold dilution to bring it to the same theoretical concentration as our 'Standard' 2.5 per cent. extract of fresh gland substance. We suggest that, at corresponding dilutions, no commercial extract should have less than 55 per cent., or more than 65 per cent., of the activity of our 'Standard' extract on the isolated guinea-pig uterus. If the activity is outside these limits, we recommend that it should be adjusted by dilution or blending to the 60 per cent. level of activity.

A further test on the blood pressure of the cat or dog is desirable, to ensure that the pressor principle has not been preferentially destroyed by some accident of preparation; but we cannot, from our own experience, recommend this test as a basis for quantitative adjustment.

With regard to the keeping qualities of the Standard extract, it will be clear, from data given above, that we found its activity not perceptibly diminished during a fortnight, or even longer, in the refrigerating chamber, after approximate sterilization in test-tubes. Our recommendation, however, is that the Standard should be freshly prepared as often as required. We do not assume that it will be necessary to make a new batch of the Standard for the assay of every batch of the commercial extract. For limited periods we believe that a comparison of each batch with its accurately assayed predecessor should suffice for the maintenance of uniformity without significant error. On the other hand, it is evident that such a process cannot be indefinitely repeated, without ultimate danger of serious departure from the original standard of activity, in one direction or the other. For this reason it is essential that there should be an independent Standard, which can be reproduced at any time, and used as a check against the development of different standards by the different makers, which the use of the batch-to-batch method alone would inevitably entail.

If the procedure which we have indicated is conscientiously followed, we believe that there should be no real difficulty in the maintenance of a practical uniformity among the pituitary extracts produced by all manufacturers who have the requisite equipment and staff. When once that is secured, it will be possible to deal with the urgent question of therapeutic dosage. From the first

introduction of the extract into practice the information on this important point has been of the most unsatisfactory kind. The tendency of the practitioner, perhaps naturally, is to inject the whole contents of an ampoule, in any case in which the administration of the pituitary extract is indicated. There is an obvious possibility that a dose which is safe and necessary in one condition may be extremely dangerous in another; but it is useless even to discuss the question of dosage, when a dose, apparently the same as another, may be anything up to 80 times as powerful.

We understand that a committee appointed by the Obstetrical Section of the Royal Society of Medicine, with the co-operation of the Medical Research Council, is conducting a thorough investigation into this question of the doses appropriate to different conditions. It will be truly lamentable if the results of this important inquiry, when in due course they are published, should be deprived of all accurate meaning by the failure of the manufacturers to bring all the makes of pituitary extract to such a uniform standard of activity as, without serious trouble, could be ensured.

We wish to thank our colleague, Dr. H. W. Dudley, who has prepared for us the majority of the pituitary extracts used in the above investigation, and also Miss F. M. Durham, who has carried out several of the tests.

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